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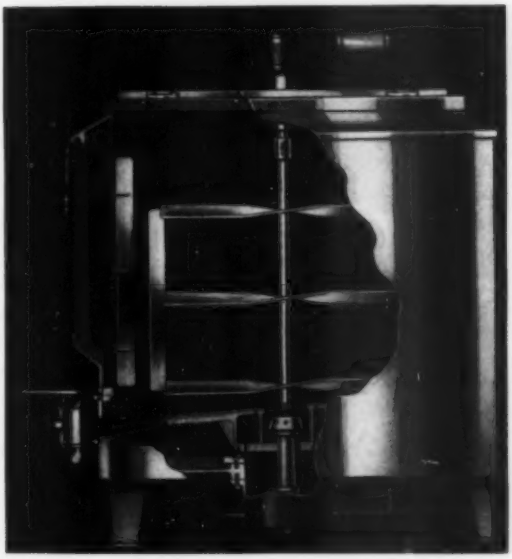

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BOUND WATER AND ITS RELATION TO SOME DAIRY PRODUCTS

I. THE BOUND WATER CONTENT OF SOME MILK PRODUCTS AND OTHER PRODUCTS USED IN THE DAIRY INDUSTRY[1]

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It has been thought for many years that proteins and some other substances owe their stability in milk totally or in part to the degree to which they are hydrated. Some explanations offered to account for many dairy phenomena have been based largely upon this assumption but no quantitative data have been presented to substantiate or refute the theoretical deductions. It is becoming more and more evident that the study of the colloidal state will clarify some of the phenomena that exist in living structures as well as in inanimate colloidal systems like milk and dairy products. In the fields of medicine, biology, physiology, agronomy; biological, physical and industrial chemistry; ceramics, geology, and agriculture, certain phenomena are explained on the basis of hydration of the colloids. It is possible that this colloidal phenomenon may play a part in certain physico-chemical properties of dairy products.

If the fluid dairy product in question lacks colloidal stability, in many cases partial precipitation of the proteins occurs. It is possible that some of the defects that occur in ice cream, milk, evaporated milk, sterilized cream, etc., may be associated with the ability of the milk proteins to bind water or to readsorb water after certain treatments.

Certain substances in the colloidal state have a tendency to hold a quantity of a given liquid with great force. This attraction when exhibited toward water, results in a hydration of the colloidal particles and gives rise to the phenomenon known as water-binding. Water-binding or hydration is usually accompanied with certain physical and chemical changes in the colloidal system. In the case of dairy products it is found that the viscosity may increase, the alcohol stability of the proteins may increase, and a portion of the water in the system will no longer act as a solvent. "Bound Water" in

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[1] Authorized for publication on December 7, 1937, as paper No. 804 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] The data presented in this paper are from a thesis submitted to the Graduate School of The Pennsylvania State College in partial fulfillment of the degree of Doctor of Philosophy, 1937.

this paper is considered to be that water which will not dissolve sucrose and is the term applied to water held on the surface of colloidal particles, water of hydration, etc. Bound water represents the difference between the total water and the "free" water contained in a system. It is important to recognize that the method used in determining bound water makes an arbitrary division somewhere between water firmly held and that loosely held and that water that is held to such a degree as not to dissolve sucrose is designated as "bound water."

HISTORICAL

The specific literature pertaining to the bound water of dairy products is meager. The literature dealing with bound water in other biological systems is much more extensive. In a paper of this nature it would be impractical to cover everything that has been written on bound water as related to other fields. Consequently the literature cited on the bound water of dairy products will be reviewed thoroughly.

Dahlberg (1) tried several methods of establishing the presence of water held physically or chemically in ice cream mixes so that it could not form large ice crystals. The freezing point method which he used is as follows: "Sugar was added to pure water and ice cream mixtures so that the percentage of sugar added when calculated on the water basis was the same in both cases. The sugar dissolved in the same concentration in both cases because the same decreases in freezing points were obtained by the Hortvet cryoscope. This experiment was repeated after the ice cream and water had been set with gelatin in the hope that the gelatin would so retard the diffusion of the sugar that any fixed water would be comparatively free from sugar when the freezing point readings were made. Although differences in the depression of the freezing points were obtained, they were less than 0.01° C., and were considered insignificant."

Caulfield (2) made bound water determinations on ice cream mixes using the freezing point technique. The milk solids-not-fat were furnished from condensed skimmilk. He reported the percentages of bound water ranging from 8.02 to 8.19 per cent.

Hinkley (3) recalculated Caulfield's data using the correction factor found for the Beckman thermometer used, and the molecular depression constant (2.268° C.) that was obtained. The bound water content was then found to be somewhat lower.

Walts (4), also using the cryoscopic method, determined the bound water content of various dairy products. With a mixture of milk plasma containing 15.17 per cent solids-not-fat he obtained a value of 12.11 per cent bound water after aging at 40° F. for 4 hours. He showed that heating milk to pasteurization temperatures and above, lowers the bound water content.

Horvat and Wright (5) determined the bound water content on a mixture containing 65 per cent milk solids, and concluded that, "There is no

evidence of the existence of 'bound' water in solutions of milk solids up to concentrations equivalent to 65 grams solids per 100 grams water."

Mumm (6) studied the hydration of cheese curd using filtration and centrifugal methods and found that when the temperature of determination was varied, a decreased hydration of the casein occurred with increased temperature on acidified and naturally soured milk. In general, increased hydration of the casein occurred on aging. Previously heated milk yielded higher curd volumes than the raw milk. The hydration of albumin was found to decrease with an increase in temperature, but this effect was not as pronounced as in the case of casein. All the salts tried had some effect on the hydration of the casein. Whether an increase or decrease occurred, depends on the pH of the solution.

McDowall and Dolby (7) while working with cheddar cheese noted that a sharp fall in the lactose content of the whey occurs when salt is added to the curd, at whatever stage the salt is added, showing that bound water is liberated. After the curd is cut, lactose is more concentrated in the whey than in the original milk. They calculate that about 2 per cent of the water of milk is bound, *i.e.*, the amount of bound water in cheese curd is about four-fifths (80 per cent) of its casein content. Later McDowall and Dolby (8), with additional evidence, indicate that 0.2–0.3 gram of water per gram of dry matter in the curd is bound.

EXPERIMENTAL METHODS

The milk and dairy products used in this investigation were obtained from the College Creamery. The same lot of spray process dry skimmilk was used throughout the investigation.

The "milk plasma mixes" were made from spray process dry skimmilk and distilled water to represent the entire serum solids content of an ice cream mix containing 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, 0.30 per cent gelatin and 62.7 per cent water. These mixes contained then, 10 per cent serum solids from spray process dry skimmilk and 62.7 per cent distilled water, the other ingredients being omitted, because of the difficulty of determining the bound water content in the presence of butterfat, sugar and gelatin. The standard procedure was to pasteurize the mixes at 143° F. for 30 minutes and cool to 40° F. in ice water.

Total solid determinations were obtained in duplicate by the Mojonnier method, titratable acidity according to the method of the A. O. A. C. (9), and pH with the quinhydrone electrode.

Protein stability was determined by the use of the alcohol number method. The test is made by noting the least amount of 95 per cent ethyl alcohol necessary to produce an evident feathering in 5 ml. of the sample placed in a test tube and treated first with water and then alcohol, always arranging the additions so that the water and the alcohol equaled 10 ml. The tubes

were inverted several times. Fine particles of curd visible on the inside surface of the test tube indicated the point of destabilization.

The viscosity was measured with a MacMichael viscosimeter, using the disc bob and a No. 27 wire calibrated with standard sucrose solution. These determinations were made in triplicate at exactly 20° C.

The freezing point determinations were made with a Hortvet cryoscope equipped with an improved standardized Beckman thermometer having a 5° C. temperature range graduated to one-hundredth of a degree. The procedure used for the freezing point determinations was as outlined in The Standard Methods of Milk Analysis (10) except that super-cooling was usually $1° \pm 0.1°$ C.

The sugar used in the bound water determinations was Sucrose C.P. "Pfanstiehl" with a specific rotation of $+66.5°$ and a moisture content of 0.1 per cent. The experimental freezing point depression of 342.24 grams of sucrose dissolved in 1000 grams of pure water was always found to be slightly in excess of that expected for the theoretical depression of 2.085° C. The values obtained, therefore, have been used in this investigation rather than using the theoretical depression or those given by other authors.

The procedure used in making the bound water determinations is in general the same as that described by Newton and Gortner (11). Modifications and a detailed account of the method used are as follows: The freezing point depression of the sample was obtained by the use of the Hortvet cryoscope. Corrections for under-cooling were made by the use of the table given by Harris (12).

The total solid content having been previously obtained by the Mojonnier method, a portion of the sample being investigated, containing exactly 100 grams of water, was weighed out. This was a convenient quantity to permit the determination of the freezing point in duplicate or triplicate, if necessary. The same portion of the sample was never used twice. The sample must be weighed accurately as the principle of the method depends upon the determination of small differences in the amount of free water present in the sample. To the weighed portion of the sample were added 34.224 grams of pulverized C.P. Sucrose, just sufficient to make a molar concentration in the total water present. (It was later found that adding the sample to the weighed beaker and sugar gave better results.) The sucrose samples were previously weighed into small glass beakers and stored in a desiccator over sulfuric acid until required. Twenty minutes of frequent stirring with a glass rod was used before the freezing point of the solution was determined. The sucrose had been ground in a Wiley mill so that it would dissolve more readily.

The freezing point depression was again obtained on the solution containing the sucrose and the correction for under-cooling again applied. The

depression was usually found to be greater than the sum of the values for the sample and the sugar solution when determined separately.

It is assumed from these results that not all of the calculated 100 grams of water in the sample is in the free state, a certain quantity being held as "bound" water by colloids present. The amount of water available for dissolving the sucrose is equal to 100 grams less the quantity "bound." The molar concentration of sugar is, therefore, increased more than the theoretical amount, and this will be shown by an excess depression of the freezing point. The magnitude of the excess depression is taken as a measure of the water held in such a way as not to be available for the solution of sucrose which is designated in this paper as bound water.

The formula of Newton and Gortner (11) used to calculate the per cent of bound water is as follows:

$$\text{Per cent Bound Water} = \frac{\Delta_x \times 89.2}{\Delta_s}$$

Where:

Δ = the freezing point depression of the sample.

Δ_s = the freezing point depression of the sample after adding 34.224 grams of sucrose (1/10 mole.) to a quantity of sample containing 100 grams of water.

$\Delta_s = \Delta_s - \Delta$ (the actual depression of the freezing point due to sucrose).

$\Delta_x = \Delta_s - 2.148$,[3] the amount (determined experimentally) by which the depression, found on addition of the sucrose is in excess of that expected over the theoretical depression.

89.2 = one mole. hexahydrate dissolved in $1000 - (18 \times 6)$ or 892 grams of water. Since 100 grams of water are used the factor becomes 89.2 in the one-tenth molar solution of sucrose.

The Bound Water Content of Milk and Some Milk Products

Some earlier work by Caulfield (2), Hinkley (3) and Walts (4) indicated that milk and milk products are hydrophilic in nature and that the cryoscopic method could be used to measure the bound water content of liquid dairy products.

To determine if milk and milk products contain appreciable amounts of bound water, several samples were obtained and the bound water content determined. The amount of water bound depends on the total solids content and such factors as salt balance, acidity, and protein stability, either natural or acquired through some previous treatment. Consequently, two samples having the same solids content may vary in bound water content. Bound water content was determined on milk, skimmilk, cream, buttermilk, condensed skimmilk, and colostrum milk. These data together with other studies on viscosity, and protein stability, are given in Table 1.

[3] In other lots of sucrose this figure becomes 2.132, 2.165 and 2.160 respectively.

TABLE 1

The bound water content of some milk and milk products

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol number	Per cent bound water
1. Raw Milk	24	13.25	6.49	2.387	0.205	8.2	3.18
2. Skimmilk	24	9.44	6.51	1.877	0.210	8.5	2.13
3. Cream	8	29.08	6.66	8.928	0.145	.....	2.50
4. Cream	8	43.20	6.73	31.683	0.135	.....	3.42
5. Buttermilk	0	8.25	5.70	1.797	0.290	3.0	1.75
6. Cond. Skimmilk	24	25.33	6.15	7.650	0.580	5.4	11.62
7. Colostrum	24	19.17	6.12	11.120	0.330	5.2	4.65

Throughout this investigation it was found that normal whole milk binds approximately 2 to 3.5 per cent water. Skimmilk binds less water than whole milk, the reason being, as will be later shown, that the fat globule "membrane" is hydrophilic. Cream has associated with it some bound water and it will be noticed that as the fat content increases the bound water content also increases. Sweet cream buttermilk, on the average, binds slightly less water than does skimmilk. Condensed skimmilk, since it is concentrated, binds relatively a large percentage of water.

Water Bound by Some of the Constituents of Milk

The substances in milk thought to have hydrophilic properties are casein, lactalbumin, globulin, and colloidal di-calcium phosphate. It has been found in this work that the fat globule "membrane" also binds a portion of the water in milk. This study, therefore, was undertaken to determine what substances in milk bind water and to what extent this water is bound by these substances.

A fresh sample of whole mixed milk from the college herd was used to determine the relative amounts of water bound by each of the constituents. The milk was divided into five lots as follows: (1) fresh whole milk; (2) skimmilk obtained by separating the whole milk; (3) skimmilk treated with rennet to coagulate the casein and to obtain the serum, and the pH regulated with sodium bicarbonate; (4) same as (3) except that the serum was brought to a pH 4.7 with acetic acid; and (5) serum at a pH 4.7 boiled to precipitate the albumin and then filtered. This milk contained 12.10 per cent total solids, 8.85 per cent plasma solids, 3.25 per cent fat, 2.34 per cent casein, 0.65 per cent albumin, and 5.86 per cent solids remaining including lactose, salts and soluble proteins. From the data obtained it was possible to calculate the amount of water bound by each of the above constituents in the concentrations as they appear in milk. The results of this experiment are given in Table 2.

TABLE 2

The water bound by the constituents of milk (rennet coagulation)

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol No.	Per cent bound water	Grams bound water per gram solids
1. Whole Milk	4	12.10	6.56	2.352	0.165	8.3	2.88	0.238
	24	12.10	6.56	2.382	0.170	8.4	2.92	0.241
2. Skimmilk	4	8.85	6.57	2.088	0.175	8.4	2.58	0.291
	24	8.85	6.57	2.088	0.180	8.5	2.58	0.291
3. Serum at pH 6.58	4	6.51	6.51	1.794	0.105	.....	0.89	0.136
	24	6.51	6.52	1.794	0.105	.....	0.97	0.149
4. Serum at pH 4.76	4	6.40	4.76	1.705	0.245	.....	0.44	0.068
	24	6.40	4.75	1.705	0.250	.....	0.44	0.068
5. Remaining Solids	4	5.86	6.23	1.617	0.095	.....	0.12	0.020
	24	5.86	6.25	1.617	0.100	.....	0.12	0.020

The data in Table 2 show that bound water decreases when the colloidal material is removed from the milk. That is, the data show that on the removal of the hydrophilic colloids by precipitation and filtration the excess depression of the freezing point becomes less and less until it becomes practically nil in sample No. 5. This, consequently, gives proof that emulsoid colloids do in fact bind part of the water.

From these results it was found that milk containing 2.92 per cent bound water after 24 hours aging at 40° F. had a distribution of its bound water content as recorded in Table 3.

TABLE 3

Water bound by the constituents of milk expressed on gram and percentage basis (rennet coagulation)

Constituent	Per cent in milk	Grams bound water per gram material	Percentage of the water bound
1. Casein	2.34	0.688	55.14
2. Albumin	0.65	1.30	29.11
3. Fat + Butterfat Membrane	3.25	0.104	11.65
4. Remaining Solids	5.86	0.020	4.10

It may be seen that casein binds about 55 per cent of the water that is bound in milk, albumin approximately 29 per cent and the fat plus the "membrane" in the concentration found in milk 11.65 per cent, while the remaining solids which consist of lactose, minerals and possibly a small amount of colloidal material binds 4.10 per cent of the bound water.

On a gram basis it is noticed that the albumin is more hydrophilic than the casein and the fat plus the "membrane" is less hydrophilic than either the casein or the albumin. Nevertheless, it will be shown later that the fat globule "membrane" when isolated from the fat globules binds approximately the same amount as the casein.

The data in Table 2 also show that at a pH of 6.52 the serum binds approximately twice as much water as it does at a pH of 4.75. This shows that as the isoelectric zone is reached less water is bound by the albumin, but precipitation does not occur unless further dehydration by alcohol or heat is practiced.

Another experiment was conducted to determine if precipitation with acetic acid gave any different results than precipitation with rennet on the water bound by the constituents of milk. The pH was also adjusted where necessary with sodium bicarbonate. The data obtained are given in Table 4.

The data in Table 4 when analyzed show practically the same results as outlined previously with rennet coagulation. Although the milk was higher in total solids content the amount of water bound by this sample was practically the same as in the previous experiment.

TABLE 4

The water bound by the constituents of milk (acid coagulation)

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Alcohol No.	Per cent bound water	Grams bound water per gram solids
1. Whole Milk	4	13.72	6.59	2.382	0.195	8.6	2.60	0.189
	24	13.72	6.58	2.441	0.195	8.7	2.91	0.212
2. Skimmilk	4	9.12	6.60	2.058	0.205	8.7	2.29	0.251
	24	9.12	6.60	2.117	0.205	8.7	2.37	0.259
3. Serum at pH 6.5	4	6.72	6.58	1.798	0.18	.....	0.90	0.133
	24	6.72	6.58	1.798	0.18	.....	0.94	0.139
4. Serum at pH 4.7	4	6.64	4.76	1.718	0.46	.....	0.39	0.058
	24	6.64	4.76	1.718	0.46	.....	0.39	0.058
5. Remaining Solids	4	6.07	6.39	1.624	0.17	.....	0.08	0.013
	24	6.07	6.40	1.624	0.17	.....	0.08	0.013

The analysis of the milk together with the amount of water bound on a gram and percentage basis is given in Table 5.

TABLE 5

Water bound by the constituents of milk expressed on gram and percentage basis (acid coagulation)

Constituent	Per cent in milk	Grams bound water per gram material	Percentage of the water bound
1. Casein	2.40	0.596	49.10
2. Albumin	0.65	1.32	29.55
3. Fat + Butterfat Membrane	4.60	0.117	18.55
4. Remaining Solids	6.07	0.013	2.74

From the results of analysis this milk contained 13.72 per cent of total solids, 9.12 per cent plasma solids, and 4.60 per cent fat.

A comparison with the results in Table 3 show that with the greater percentage of fat there is more water bound since there is more "membrane" material present.

The results of these experiments show that casein binds approximately 50 per cent of the water that is bound in milk. The casein content of normal cow's milk varies between 2.25 per cent and 2.75 per cent and since it is present in greater quantities than any of the other colloid substances, it probably plays the greatest role in the colloidal properties of milk.

The Bound Water of Whey Protein and Lactose Solutions

In Tables 3 and 5 it was shown that the albumin present in milk is about twice as hydrophilic as casein. Undoubtedly it also plays a role in the colloidal properties of dairy products but since it is more of a true hydrophilic colloid than casein, factors that may affect casein may not affect albumin.

Experiments were conducted with some whey protein powder obtained from the Bureau of Dairy Industry, U. S. D. A. This powder had been electrodialyzed and contained 6.51 per cent ash, 1.22 per cent moisture, 22.71 per cent whey protein, and 69.56 per cent lactose. At first it was thought desirable to determine the bound water of the whey protein powder in the concentration found in milk at pH values of approximately 4.8 and 6.7 and compare the results with some of the data recorded in Tables 2 and 4 with the milk serum.

Another sample of whey protein powder was dialyzed in collodion membrane bags until the tests for chlorides and lactose were negative. The distilled water was changed at least once a day. The samples were kept at a temperature of 38° F. while dialyzing and toluene was used as a preservative. The object was to obtain the whey proteins as free of salts and lactose as possible and to determine the bound water content on the purified whey proteins. To note whether lactose affected the bound water determinations, a

lactose solution was prepared and the bound water determined in the usual manner. The results of these studies are given in Table 6.

TABLE 6

The bound water content of whey protein, dialyzed whey protein, and lactose solutions

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Whey Protein Powder*	4	5.99	4.81	1.291	0.27	0.66	0.110
pH 4.82	24	4.99	4.82	1.352	0.27	0.74	0.123
2. Whey Protein Powder*	4	6.53	6.66	1.411	0.125	2.17	0.333
pH 6.67	24	6.53	6.67	1.470	0.13	2.37	0.362
3. Whey Protein Powder*	4	5.67	6.79	1.441	0.135	2.07	0.365
pH 6.78	24	5.67	6.78	1.500	0.14	2.23	0.393
4. Pure Whey Protein	1 wk.	0.28	7.58	1.147	0.00	0.21	0.750
5. Pure Whey Protein	4 da.	0.57	6.55	1.294	0.01	0.38	0.650
6. Lactose	4	4.42	5.73	1.088	0.00	0.12	0.027
	24	4.42	5.73	1.088	0.00	0.12	0.027

* This sample contains lactose and minerals of milk.

The results obtained with whey protein sols, made from a special whey powder, showed that these whey proteins bound considerably more water than the whey proteins found naturally in milk (Tables 2 and 4). There is more water bound by the whey proteins at a pH of 6.67 than at a pH of 4.82 and this would seem to indicate that at the isoelectric point there is less water bound by the whey proteins than at the normal pH of milk.

The whey powder proteins did not bind as much water as the whey protein obtained by dialyzing in a collodion bag until free from electrolytes. Possibly the explanation lies in the fact that natural sols bind more water than prepared sols as has been reported by Newton and Martin (13).

The bound water studies with lactose indicate that it does affect the bound water determinations of dairy products. The slight amount of water bound can be attributed to the experimental error of the method employed.

The Bound Water Content of Cream of Various Fat Percentages

During the course of the work on cream, it was noticed that creams of various fat percentages bound approximately the same amount of water per gram of total solids. To obtain more information, creams of approximately 18 per cent, 31 per cent, and 40 per cent fat were produced and bound water determinations made after aging 4 and 24 hours at 40° F. (Table 7).

TABLE 7

The bound water content of cream of various fat percentages

Per cent fat in cream	Hours aged	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. 18.08	4	25.79	6.59	5.735	0.14	4.71	0.182
	24	25.79	6.57	6.029	0.145	4.82	0.186
2. 30.93	4	37.25	6.65	21.176	0.12	6.88	0.187
	24	37.25	6.63	22.352	0.125	7.08	0.190
3. 40.33	4	45.68	6.72	31.176	0.105	8.59	0.188
	24	45.68	6.74	33.235	0.105	8.93	0.195

Although the 18 per cent cream contained approximately 7.71 per cent of solids-not-fat and the 40 per cent cream 5.35 per cent solids-not-fat, the bound water content per gram of total solids remained apparently the same. The explanation for this phenomenon lies in the fact that the fat globule membrane is hydrophilic to about the same degree as the casein. Later results show that this hypothesis is correct.

Perlman (14) shows that the phospholipid content of cream increases uniformly with the fat content up to approximately 55–58 per cent, after which it diminishes with further increases in fat content. He also shows that the phospholipid content per unit of fat remains fairly uniform in cream containing between 20 per cent and 60 per cent fat. It can be concluded from the data that on an equal solid basis creams of various fat percentages in the range studied will bind approximately the same amount of water per gram of solids.

The Bound Water Content of the Fat Globule Membrane and Pure Milk and Egg Phospholipids

In this study the fat globule "membrane" was obtained according to the method of Palmer and Wiese (15). The isolation of pure milk and egg phospholipids was based on the procedure outlined by Bull (16) and noted by Josephson and Dahle (17).

The pure milk phospholipids, even though waxy and fat-like in appearance, disperse uniformly in water to form a cloudy viscous sol. Bound water determinations were made on sols of the fat globule "membrane," pure milk and egg phospholipids. The results obtained are recorded in Table 8.

From the results obtained in Table 8, pure milk phospholipids appear to be more hydrophilic than any substance studied. A sol of the milk phospholipids containing 0.63 per cent solids bound 6.46 grams of water per gram of solids after 24 hours aging. It is apparent that the bound water content of the fat globule membrane is almost a linear function of concentration. It will be remembered similar results were obtained with cream (Table 7) where

TABLE 8

The bound water content of fat globule membrane and pure milk and egg phospholipids

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Fat Globule Membrane	4	2.83	6.54	1.470	0.04	1.63	0.58
	24	2.83	6.54	1.614	0.04	1.79	0.63
2. Conc. Fat Globule Membrane	4	10.60	6.91	6.117	0.095	5.65	0.53
	24	10.60	6.90	6.264	0.10	6.33	0.59
3. Pure Milk Phospholipids	4	0.63	6.76	2.294	0.01	3.88	6.16
	24	0.63	6.74	2.353	0.01	4.05	6.46
4. Pure Egg Phospholipids	4	1.08	6.23	2.353	0.01	1.93	1.79
	24	1.08	6.23	2.382	0.01	2.05	1.90

the bound water content was attributed to the hydrophilic properties of the fat globule membrane.

Pure egg phospholipids bound relatively less water per gram solids than pure milk phospholipids. A sol containing 1.08 per cent of total solids showed after 24 hours aging 1.90 grams of bound water for each gram of solids. It is possible that some of the original properties of the egg phospholipids may have been altered as the material was prepared from dried egg yolk. There is also a marked difference in certain other properties of the phospholipids obtained from different sources as Jack (18) found the isoelectric point of milk phospholipids to be pH 2.0, while Price (19) found the isoelectric point of egg lecithin to be pH 2.7.

On an equal solids basis it seems that the viscosity of the milk phospholipids is greater than that of the egg phospholipids. From the bound water results obtained this would seem to be correct as the milk phospholipids are more hydrated. The pH of milk phospholipids was slightly lower than that obtained with egg phospholipids.

The Bound Water Content of Some Gums and Stabilizers Used in the Dairy Industry

In the past, and also to some extent at present, gums have been used as stabilizers in ice cream, ices and sherbets, because they have the property of absorbing water and the amount absorbed may be many times their weight. Some of these gums and stabilizers form gels at higher concentration, while others remain as sols. Their action in ice cream, ices and sherbets is to produce a finer texture by preventing formation of large ice crystals in the frozen product.

Studies were made on gum arabic, oat flour, egg yolk, locust bean flour, "colace," gelatin and sodium alginate sols.

Gum arabic, although not used extensively in dairy products, was used to study the effect of concentration on the bound water content since it does

not gel upon increasing the concentration. Approximately 1.4 and 8 per cent sols were made and compared.

Another experiment was conducted with oat flour (Avenex No. 7) to determine whether this material is hydrophilic and also to determine the effect of concentration on the bound water content. Oat flour has antioxygenic properties and when used in ice cream also acts as a stabilizer. Oat flour mixtures were made so that water extract solutions of approximately 0.5, 1.0 and 2.0 per cent resulted.

The bound water content of egg yolk, locust bean flour and "colace" was determined on approximately one per cent sols.

Approximately 0.5 per cent gelatin sols were prepared from 175, 200 and 250 Bloom gelatins to study the hydrophilic properties of this stabilizer. Three different concentrations of sodium alginate were also studied and these results together with those of gum arabic, oat flour, egg yolk, locust bean gum, and "colace" are given in Table 9.

TABLE 9

The bound water content of some gums and stabilizers used in the dairy industry

Sample	Hours aged at 40° F.	Per cent solids	pH	Viscosity centipoises	Per cent acid	Per cent bound water	Grams bound water per gram solids
1. Gum Arabic	4	0.93	4.90	2.054	.....	0.37	0.40
	24	0.93	4.86	2.054	.....	0.37	0.40
2. Gum Arabic	4	4.32	4.54	4.701	.....	2.36	0.55
	24	4.32	4.54	4.701	.....	2.39	0.55
3. Gum Arabic	4	8.15	4.39	8.533	.....	4.81	0.59
	24	8.15	4.39	8.236	.....	4.72	0.58
4. Oat Flour Extract	4	0.49	6.29	1.041	0.01	0.12	0.241
	24	0.49	6.31	1.041	0.01	0.16	0.326
5. Oat Flour Extract	4	1.23	6.32	1.143	0.015	0.24	0.195
	24	1.23	6.36	1.143	0.015	0.32	0.260
6. Oat Flour Extract	4	2.00	6.34	1.388	0.02	0.45	0.225
	24	2.00	6.36	1.388	0.02	0.49	0.245
7. Dried Egg Yolk	4	1.18	5.51	1.294	0.03	0.89	0.75
	24	1.18	5.53	1.323	0.03	1.09	0.92
8. Locust Bean Flour	4	1.05	5.06	132.352	0.005	1.40	1.33
	24	1.05	5.04	135.294	0.005	2.03	1.93
9. "Colace"	4	1.22	4.55	100.588	0.005	0.86	0.70
	24	1.22	4.56	102.941	0.005	1.12	0.91
10. Gelatin 175 Bloom	4	0.50	4.24	1.481	.....	− 0.31	.....
	24	0.50	4.26	2.212	.....	− 0.20	.....
11. Gelatin 200 Bloom	4	0.57	4.41	2.054	.....	− 0.56	.....
	24	0.57	4.39	3.081	.....	− 0.31	.....
12. Gelatin 250 Bloom	4	0.57	4.44	3.239	.....	− 0.52	.....
	24	0.57	4.54	11.535	.....	− 0.07	.....
13. Sodium Alginate	4	0.59	8.64	9.411	.....	− 1.61	.....
	24	0.59	8.64	9.411	.....	− 1.61	.....
14. Sodium Alginate	4	1.36	9.11	39.411	.....	0.33	0.242
	24	1.36	9.11	39.705	.....	0.33	0.242
15. Sodium Alginate	4	2.53	9.30	119.117	.....	0.65	0.257
	24	2.53	9.30	120.000	.....	0.65	0.257

With most colloids the bound water per gram of colloid tends to diminish with the concentration of the sols. Gum arabic seems to be an exception. Over the range studied bound water appears to be almost a linear function of concentration. This characteristic does not hold for all gum arabic. Newton and Gortner (11) found, in their original experiments on which the cryoscopic method of determining bound water was based, that there is a logarithmic relation between bound water content and concentration in this gum arabic. Later Newton and Martin (13) obtained results to show that bound water is almost a linear function of concentration with some gum arabic.

Samples numbers 4, 5 and 6 show that the water extract of oat flour is not very hydrophilic and binds a relatively small amount of water per gram of extractable material. Nevertheless, the relative insolubility of oat flour should not prevent it from binding water. The non-colloidal fraction, therefore, may be responsible for the stabilizing properties of oat flour. Newton and Martin (13) show that although gelatin, agar, and blood fibrin are relatively difficult to disperse in cold water, they bound more water than substances colloidally dissolved.

The results show that egg yolk, locust bean flour and "colace," which contains locust bean flour, are quite hydrophilic in nature and some of the properties they impart to ice cream are probably due to this factor. The viscosities of the sols of locust bean flour and "colace" are very great which is typical of most gums and stabilizers.

From the results obtained with gelatin and sodium alginate in Table 9, it is apparent that the cryoscopic method cannot be used to determine the bound water content of substances of this nature, since the water held by these substances will dissolve sucrose. In fact, in most instances, negative amounts of bound water were obtained. It is possible that the structure of these sols is different from other sols, or a slight positive adsorption of the sucrose would account for these findings; that no water was "bound."

Sayre (20) points out that, "If water held by gelatin and agar is considered as bound water, then this method fails to give a real measure of bound water, since much of the water held by those substances will dissolve sucrose." Nevertheless, Versmold (21), Newton and Martin (13) and others report the presence of bound water in agar and gelatin when the cryoscopic technique is used.

These data bring out the fact that gelatin increases in viscosity on aging while sodium alginate does not and this correlates with the fact that ice cream mixes made with sodium alginate also do not increase noticeably in viscosity on aging, while gelatin mixes usually do.

SUMMARY AND CONCLUSIONS

The cryoscopic method can be readily used for the determination of bound water in liquid dairy products with few exceptions.

Milk, cream and other liquid dairy products contain appreciable amounts of bound water. In a milk of average fat and total solids content, the casein binds approximately 50 per cent of the bound water, the albumin 30 per cent, the fat globule "membrane" 15 per cent, and the remaining solids less than 4 per cent.

There is some indication that rennet casein binds more water than acid casein and therefore this fact may contribute to some of the differences of the two caseins.

More water is bound by the true hydrophilic colloids (whey proteins) of milk at a pH 6.6 than at a pH 4.7. Dialyzed whey protein bind as much water as natural whey proteins found in milk. Lactose does not affect bound water determinations of liquid dairy products with the cryoscopic method.

A large portion of the bound water present in cream is due to the hydrophilic properties of the fat globule "membrane" and in cream containing between 18 and 40 per cent butterfat the bound water content per gram of solids remains fairly constant.

The fat globule membrane binds approximately the same amount of water as the casein. Pure milk phospholipids are found to be the most hydrophilic of any substances isolated from milk in this study. Pure egg phospholipids are less hydrophilic than the pure milk phospholipids.

In this study the bound water content of gum arabic appears to be almost a linear function of concentration. The colloidal extract of oat flour (Avenex No. 7) is not very hydrophilic. Powdered egg yolk, locust bean flour and "colace" bind relatively large quantities of water while gelatin and sodium alginate give negative results, in some instances, with the cryoscopic method.

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BACTERIAL CONTENT AND KEEPING QUALITY OF BUTTER AFTER REMOVAL FROM STORAGE

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Recent practices in butter storage plants have tended toward lower holding temperatures and in some cases temperatures considerably below freezing have been employed. The present investigation was outlined to study the influence of freezing on the types and numbers of bacteria in butter and also to follow the subsequent changes in flavor and bacterial content when the butter was removed from frozen storage. The practical value of holding butter below freezing depends not only on the keeping quality protection during holding but also on the influence of such holding on the keeping quality of the frozen butter after it has reached the distributor, retailer or consumer.

REVIEW OF LITERATURE

Only a few reports from the extensive literature on the influence of low temperature storage on butter will be included here. A more inclusive review has been published by the author (2) in a previous paper.

In 1906, Gray and McKay (3) proved that butter held at −10° F. kept better both while in cold storage and after removal from storage than when stored at higher temperatures. Rogers and coworkers (4) obtained similar results and indicated that the difference between the results at −10° F. and 0° F. was enough to warrant the use of the lower temperature.

The changes in the bacterial content of butter stored below freezing have been studied by a number of investigators. In many cases the results have shown no significant changes, although the tendency has been for decreases in counts when temperatures much below the freezing point were used. Washburn and Dahlberg (5) concluded that bacteria in unsalted butter decreased more rapidly at −15° F. than they did in salted butter, but little if any relationship was noted between the numbers of bacteria and the butter score after storage. Grimes (6) held salted butter from ripened and unripened cream for a period of six months at −6° F. and found that marked decreases in counts occurred, the greatest decrease resulting in the ripened cream butter. Grimes and Hennerty (7) studied the changes in numbers of microorganisms in sweet cream, salted butter held for periods of from two to eight months at 15° F. The results showed no significant change in bac-

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terial counts, an increase in yeast and a definite decrease in mold counts. No definite relationship could be noted between the changes in numbers of bacteria, yeasts or molds and the change in butter score during storage.

Since they found no significant changes in yeast, mold, or bacterial counts in butter held at −7° C. (19° F.) for six months, Arup and Gilmore (8) concluded that the flavor deterioration which resulted was of chemical origin. Loftus-Hills, Scharp and Bellair (9) arrived at similar conclusions on salted butter stored at 12° F.

METHODS OF PROCEDURE

The trials include fifteen churnings from which both salted and unsalted butter were obtained. The portions of salted and unsalted butter were each divided into two portions and placed in five-ounce glass jars, one of which was placed at −25° C. and the other held at room temperature (21° C.). Plate counts were made on the fresh butter, on the samples held at −25° C. after one day and 90 days, and on the samples held at room temperature after 2, 4 and 7 days.

After the butter had been held for 90 days at −25° C. it was held for an additional 7 days at room temperature and bacterial counts and flavor examinations were made after 2, 4 and 7 days. The results on this butter were used for comparison with the results obtained on the corresponding butter held 7 days at room temperature when fresh.

The portions for analysis were obtained from the surface and subsurface of the samples by means of a sterile spatula. The numbers of total, lipolytic and proteolytic bacteria were determined by the methods recommended by the committee on Bacteriological Methods (1). The total bacterial counts were made on beef infusion agar while the lipolytic and proteolytic bacteria were determined on a special plating using the beef infusion agar with oil emulsion, Nile blue sulphate solution and sterile skimmilk added.

Flavor examinations were made on the butter when fresh, and after holding under the various conditions described above. The butter was not scored but was described as satisfactory or defective in flavor and comments were recorded on the types of flavors detected.

The "room temperature" designation is used in the tables and discussion to indicate a temperature within ± 1° of 21° C.

RESULTS AND DISCUSSION

Changes in Numbers of Bacteria in Butter Held at −25° C.

The results given in Table 1 indicate that freezing effected a significant reduction in the numbers of bacteria in both salted and unsalted butter. The reduction in numbers during holding was much greater in the unsalted butter than in the salted butter even though the counts in the salted butter were

TABLE 1

Influence of holding at −25° C. on the bacterial counts of salted and unsalted butter

Held at −25° C.	Numbers of bacteria per ml., geometric means of 15 lots of	
	Unsalted butter	Salted butter
0 days	877,000	161,000
1 day	167,000	101,000
90 days	65,000	29,400

lower at the end of the holding period. These reports agree in general with those of Washburn and Dahlberg (5) who found more rapid decreases in counts in unsalted than in salted butter held at −15° F. (−26° C.)

The marked difference between the bacterial counts on the salted and unsalted butter when fresh was due undoubtedly to the destructive action of salt. The comparatively small decrease in the numbers in the salted butter after holding at −25° C., however, indicated that the salt effected some protective influence at the low temperature and prevented the destruction of as large a percentage of the bacteria as were destroyed in the unsalted butter. The freezing point lowering due to the salt brine in the butter serum appears as the best explanation of the protective action to the bacterial flora in the salted butter. The presence of salt may have prevented crushing to some extent by interfering with the complete crystallization of the moisture in the butter. The salt brine concentration in the butter was approximately 16 per cent and a brine of this concentration has a freezing point of −12.2 C.

Lipolytic and proteolytic bacteria were not detected on the plates poured from the butter after 1 day at −25° C. even though dilutions as low as 1 : 100 were regularly made. These results indicate that the small numbers of lipolytic and proteolytic bacteria present in the freshly made butter were inactivated or destroyed in the freezing process.

Bacterial Counts in Fresh and Storage Butter Held at Room Temperature

Table 2 presents a summary of the changes in numbers of bacteria in butter held at room temperature after storage at −25° C. for 90 days and in the corresponding butter held at room temperature when fresh. The numbers of bacteria increased more rapidly in the unsalted storage butter than in the corresponding fresh unsalted butter when held at room temperature. The bacterial counts on the two types of butter were approximately the same after the 7 days, even though the initial counts on the storage butter were comparatively lower. The numbers of bacteria, decreased in both the fresh and storage salted butter when held at room temperature and were somewhat lower on the storage butter after 7 days.

TABLE 2

Changes in bacterial counts on fresh butter and on storage butter held 7 days at room temperature

Butter	Number of churnings	Number of bacteria per ml., geometric means of 15 lots of			
		Unsalted butter		Salted butter	
		0 days	7 days	0 days	7 days
Fresh	15	877,000	22,500,000	161,000	50,900
Storage	15	65,000	20,300,000	29,400	22,900

Flavor Deterioration in Fresh and Storage Unsalted Butter Held at Room Temperature

A comparison of the flavor deterioration in 15 lots of fresh and 15 lots of storage, unsalted butter held at room temperature is presented in Table 3. Since no off-flavors were noted previous to the 4-day examination, the table gives the data for only the 4- and 7-day examinations. A flavor defect developed in only one lot of the fresh butter during the first 4 days, while six lots of the storage butter showed some flavor deterioration at 4 days. After 7 days there was no significant difference in the number of lots of fresh and of storage butter showing flavor defects, but the flavor defects were more pronounced in the storage butter than they were in the fresh butter. Flavor defects were detected after 7 days in certain lots of fresh butter when they were not detected in the corresponding storage lots, and vice versa. The

TABLE 3

Flavor deterioration in fresh and in storage unsalted butter held 7 days at room temperature

Churning No.	Flavor comments			
	After 4 days holding		After 7 days holding	
	Fresh butter	Storage butter	Fresh butter	Storage butter
1	– – –	– – –	– – –	– – –
2	– – –	sl. off	– – –	Roquefort
3	– – –	sl. off	sl. off	sl. off
4	sl. rancid	oily	rancid	sl. bitter
5	– – –	– – –	rancid	– – –
6	– – –	sl. off	sl. off	sl. off
7	– – –	– – –	sour	– – –
8	– – –	– – –	sl. off	– – –
9	– – –	– – –	– – –	– – –
10	– – –	– – –	– – –	Roquefort
11	– – –	– – –	sl. off	Roquefort
12	– – –	sl. oily	sl. rancid	Roquefort
13	– – –	– – –	– – –	Roquefort
14	– – –	sl. oily	sl. oily	moldy
15	– – –	– – –	– – –	sl. oily

– – – = flavor satisfactory; sl. = slightly.

TABLE 4

Lipolytic and proteolytic bacteria in unsalted butter held 7 days at room temperature after removal from storage at −25° C.

(Time flavor deterioration first noted—indicated by *)

Churning No.	Type of bacteria	Numbers of bacteria per ml.			Flavor defects
		2 days	4 days	7 days	7 days
1	Lip.	<10,000	<10,000	<10,000	
	Prot.	<10,000	<10,000	<10,000	
2	Lip.	<10,000	<10 000*	<10,000	
	Prot.	630,000	3,000,000	34,000,000	Roquefort
3	Lip.	130,000	45,000*	10,000	
	Prot.	<10,000	500,000	15,000	slightly off
4	Lip.	300,000	200,000*	400,000	
	Prot.	500,000	100,000	1,000,000	slightly bitter
5	Lip.	20,000	85,000	<10,000	
	Prot.	<10,000	<10,000	<10,000	
6	Lip.	xxx	4,300,000*	4,700,000	
	Prot.	xxx	100,000	<10,000	slightly off
7	Lip.	390,000	1,400,000	500,000	
	Prot.	15,000	1,200,000	850,000	
8	Lip.	355,000	150,000	55,000	
	Prot.	100,000	135,000	25,000	
9	Lip.	5,600,000	4,900,000	1,550,000	
	Prot.	900,000	1,900,000	1,900,000	
10	Lip.	4,500	20,000	5,000*	
	Prot.	5,000	55,000	10,000	Roquefort
11	Lip.	160,000	450,000	8,000,000*	
	Prot.	200,000	6,200,000	16,000,000	Roquefort
12	Lip.	130,000	2,200,000*	6,000,000	
	Prot.	50,000	1,600,000	7,500,000	Roquefort
13	Lip.	55,000	30,000	160,000*	
	Prot.	45,000	32,000	170,000	Roquefort
14	Lip.	7,500	<1,000*	<1,000	
	Prot.	6,500	5,000	5,000	moldy
15	Lip.	35,000	20,000	2,100,000*	
	Prot.	35,000	190,000	2,100,000	slightly oily

xxx too many to count with dilutions used.

most common defects noted were rancid and Roquefort flavors; there were two lots of fresh butter and five lots of storage butter developing one or the other of these defects. It is evident from these results that the storage butter failed to keep as well when placed at room temperature as the corresponding lots of fresh butter.

Flavor defects other than tallowiness were not developed in either the fresh or storage, salted butter during the 7 days at room temperature and consequently no comparison of keeping quality from a bacteriological standpoint could be made.

Lipolytic and Proteolytic Bacteria in Unsalted Butter Held 7 Days at Room Temperature After Removal from Storage at – 25° C.

The changes in the numbers of lipolytic and proteolytic bacteria in the 15 lots of unsalted storage butter held at room temperature are shown in Table 4. Although lipolytic and proteolytic bacteria were not detected in the platings of fresh butter there were increasingly large numbers evident in some of the lots after holding at room temperature. In the ten lots which showed flavor deterioration at 7 days, the numbers of lipolytic bacteria ranged from less than 1,000 to 8,000,000 per ml. The five lots, which kept, contained from less than 10,000 to 1,550,000 lipolytic bacteria per ml. The numbers of proteolytic bacteria in the butter which developed flavor defects ranged from 5,000 to 34,000,000 per ml. while the lots which kept ranged from less than 10,000 to 1,900,000 per ml. No correlation between the numbers of lipolytic or proteolytic bacteria and specific flavor defects could be noted. The lots which developed rancid flavor did not contain larger numbers of lipolytic bacteria than some of the lots which showed either indefinite flavor defects or no flavor defects. A similar lack of agreement existed between the numbers of proteolytic bacteria and flavor defects. Neither lipolytic nor proteolytic bacteria were detected in the salted butter held 7 days at room temperature subsequent to storage.

SUMMARY

Holding at – 25° C. effected marked decreases in the total numbers of bacteria present in both salted and unsalted butter but the decreases were much less pronounced in the salted butter than in the corresponding unsalted butter. The destructive action of salt was apparently of greater importance than freezing in reducing the numbers of bacteria in salted butter. No change in flavor was detected in the butter which had been frozen for 90 days.

The bacterial counts increased more rapidly and flavor deterioration was more rapid in the unsalted butter held at room temperature (21° C.) after storage at – 25° C. than in the fresh butter held 7 days at room temperature.

Large numbers of lipolytic and proteolytic bacteria were found in certain lots of unsalted butter after holding at room temperature but no definite correlation could be noted between the growth of these types of bacteria and the development of flavor defects.

The bacterial counts did not change significantly in salted butter held 7 days at room temperature subsequent to storage at – 25° C. Neither lipolytic

nor proteolytic bacteria were detected in the salted butter which had been held 7 days at room temperature subsequent to storage at – 25° C. Flavor defects other than tallowiness were not detected in the salted butter when it was held 7 days at room temperature subsequent to storage at – 25° C.

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A METHOD FOR THE EXTRACTION OF FAT IN ICE CREAM IN ORDER TO DETERMINE ITS PURITY[1]

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In our ice cream studies the last three years it has been necessary to extract large quantities of fat from the same sample of ice cream. At different times we have used various suggestive methods. After numerous trials, we confined our work to a modified Mojonnier method, using a large separatory funnel. In spite of its apparent simplicity, it frequently happened that undue emulsification occurred with the use of ammonium hydroxide causing, thereby, a loss of time and increasing the amount of reagents used.

Since the official methods (A. O. A. C. Fourth Edition, 1935) (1) does not contain a method for the extraction of fat applicable to ice cream, we have, therefore, attempted to devise a method for the rapid extraction of the fat in larger than test quantities. In order to overcome the tendency toward emulsification, various acids were tried in the place of the base, ammonium hydroxide. It was found that glacial acetic acid produced a more rapid uniform separation of the fat and there was no tendency toward charring as there was with sulphuric acid.

PROCEDURE

Two hundred grams of ice cream mix or melted ice cream was washed into a 500 ml. separatory funnel with about 50 ml. of warm distilled water. To this was added 30 ml. of glacial acetic acid and the contents shaken occasionally over a period of 15–20 minutes after which 100 ml. ethyl alcohol was added and again shaken until homogeneous. The stopper was loosened frequently to avoid pressure in the funnel. Then 75 ml. each of ethyl and petroleum ethers was added in order mentioned. The mixture was shaken thoroughly after each addition. This mixture was allowed to stand until the ether-fat layer had completely separated. The lower layer, *i.e.*, ice cream mix, was then drawn off into another separatory funnel, extracted again using 50 ml. of each of the two ethers. (One hundred ml. of the mixture of the recovered ethers may be used for the second extraction.) After the fat-ether layer was completely separated, the lower layer was discarded and the two-ether layers were combined in one separatory funnel. These were washed three times with distilled water, using about 150 ml. each time. The fat-

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TABLE 1

Comparison of methods for the extraction of fat from ice cream

Method	Chocolate		Vanilla		Extracted Fat % Yield		Remarks
	R.M. No.	K.Sap. No.	R.M. No.	K.Sap. No.	1st	2nd	
Acetic acid	24.53	224.2	28.45	227.8	75–80	5–8	Clear fat
Ammonium hydroxide	24.64	225.7	28.58	229.3	25–40	2–3	Clear fat
Sulphuric acid	24.74	225.3	28.71	228.9	70–75	5–7	Charred or darkened fat

ether mixture was then transferred to a 500 ml. Erlenmeyer flask and the ethers were distilled off of the fat. When most of the ethers were distilled off, the fat was filtered into a sample container using a short stem funnel. The filtering of the fat can best be done in an electric oven at 60° to 75° C. if the above distillation has been previously carried out. The filtered fat was then heated to about 95–98° C. for one hour to free it from possible traces of volatile substances.

Ice cream containing fruits should first be filtered through a gauze strainer to remove the fruit particles before washing into the separatory funnel.

RESULTS

Table 1 shows the comparison of the Reichert-Meissl and saponification numbers, expected yield and color of fat extracted from vanilla and chocolate ice creams. The results in Table 1 are the average of four samples of each type of ice cream picked at random from several samples. The expected yield of fat from the acetic acid method is about 75 per cent for the first extraction and about 5 to 8 per cent in the second extraction. The emusification of the fat was quite evident in the method where ammonium hydroxide was used as shown by the low yield of fat. It is also probable that some saponification of the fat occurred as noted by the persistent lather produced. The yield of fat with the ammonium method varied considerably with different samples. The sulphuric acid method gave a much darker fat than the other methods, probably due to the fact that the sugar in the ice cream was charred and carried over with the ether-fat mixture. The Reichert-Meissl and saponification numbers of the fat in chocolate ice creams are lower than pure butterfat since it contains cocoa fat from the chocolate or cocoa (2). It will be noted from the table that the fat constants for the three methods checked very closely.

SUMMARY

A method is given for the extraction of fat from ice cream in large quantities.

Results obtained in the extraction of fat from ice cream indicate that the acetic acid method is more economical, more fat is secured per extraction and takes less time than either the sulphuric acid or ammonium hydroxide methods.

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THE CHEMICAL COMPOSITION AND PROPERTIES OF NORMAL AND RANCID JERSEY MILK

I. CHLORIDE AND LACTOSE CONTENT

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During a study of methods of milk scoring, certain off-flavors were frequently observed in the milk of the college Jersey herd. Numerous inquiries as to the cause of undesirable flavors in milk emphasized the economic significance of the problem. A systematic study was made, therefore, of the occurrence of off-flavors in milk, for the purpose of determining the cause and means of prevention of their development.

Natural rancidity proved to be the most pronounced off-flavor encountered in the study. Approximately ten per cent of 928 samples scored for flavor were criticized as rancid. Frequently the flavor was so offensive that it made the milk unpalatable. "Rancid" milk, designated by some investigators as the "bitter milk of late lactation," was first described in detail by Eckles and Shaw (5). Koestler *et al.* (11) have described such milk as having a peculiar biting sharp after-taste which changes to a definite sharp acid-taste resembling rancid butter when allowed to stand at room temperature. The frequent occurrence of this flavor is indicated by Roadhouse and Henderson (14) in a survey of abnormal flavors occurring in milk from twelve commercial herds. Of 536 animals observed, 68 produced milk with abnormal tastes or flavors and of these, 17 gave naturally rancid milk.

Despite the interest which has been shown in the causes of the production of rancid milk, no systematic study appears to have been made of the occurrence of rancidity in the milk of the animals of a given herd, nor any comparison made of the chemical composition of rancid milk with that of normal milk produced under the same conditions. The present paper presents the chloride and lactose content of normal and rancid milk produced by Jersey cows maintained under regulated conditions. Subsequent papers deal with the fat, total solids, protein, lipase, titratable acidity and hydrogen-ion content of normal and rancid Jersey milk.

It is commonly recognized that the breed of animal and the period of lactation affect the composition of milk, yet there is a marked scarcity of data sufficiently detailed to serve as criteria of normal values for the different constituents at all stages of lactation. Allen (1), in a review of the mineral constituents and citric acid content of milk, discussed the lack of detailed data showing the influence of the period of lactation upon the mineral content of milk. He stated that although it is a well-known phe-

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nomenon that milk undergoes marked changes in composition as the period of lactation advances, the number of workers who have actually given detailed data for the analysis of milk at definite lactation periods is surprisingly small. In discussing the effect of the period of lactation upon the chloride content of milk, he refers to four investigations (8, 10, 16, 17), none of which were sufficiently detailed to be considered conclusive. Recent work has been reported by Sharp and Struble (15) and Caulfield and Riddell (3).

Lactose was considered by early workers (12, 5) as one of the least variable constituents of milk, but later work (6) showed that the lactose content of the milk of individuals may vary considerably from the average of the herd. Eckles and Shaw (7) found that the lactose of the milk of an individual animal may vary 0.5 per cent from the average lactose content, but that 90 per cent of the samples obtained during a given lactation show a variation of less than 0.2 per cent.

EXPERIMENTAL

The cows of the college Jersey herd were selected for observation. The management of the animals did not differ from the practice usual with the college herds. The animals were kept out-of-doors both day and night during the entire experiment except on stormy nights during the winter months. The same ration was fed throughout the experiment except that the cows were on pasture in the summer. On the days when milk samples were collected the cows were always kept off pasture and were allowed no hay or silage for five hours previous to milking. This precaution was necessary to avoid development of feed flavors. In addition to pasture in season, roughage was supplied by alfalfa, prairie hay and darso silage. The concentrate mixture used contained by weight 1 part of cottonseed meal, 4 parts of ground corn, and 2 parts each of ground oats and wheat bran and was fed in proportion to the milk yield.

The cows were milked at 12-hour intervals and individual samples representative of an entire milking were obtained once a week from each animal in the herd. Samples were taken at the evening milking since preliminary work showed no appreciable differences in the flavor of the morning and evening milk. They were kept in a refrigerator over night and the following morning were scored for flavor by the method previously reported by Fouts and Weaver (9).

Determinations of the chloride and lactose content of the individual samples were made over a period of 2 years. During this time, observations were made on 20 animals, not all of which were continuously available for the entire period. Of the 20 cows observed, 13 were from 2 to 4 years old, 4 were from 5 to 6 years, and 3 were from 11 to 12 years. At no time during the study were any of the animals reported by the station veterinarian to be suffering from mastitis, either in an acute or chronic form.

ANALYSIS OF SAMPLES

The chloride content of the milk was determined by the method of Van Slyke and Donleavy (18) as modified by Dennis and Sisson (4). In this method, 20 cc. of a 1.2 per cent solution of picric acid and 20 cc. of standard silver nitrate are added to 10 cc. of milk. Precipitation of the protein is instantaneous and complete, and after standing 10 minutes the liquid is poured on a chlorine-free filter. An aliquot of the clear filtrate is taken and the excess of silver is titrated with potassium iodide solution.

In the analysis for lactose, the protein of milk was precipitated with copper sulphate and sodium hydroxide as described in the Methods of Analysis of the A. O. A. C. Precipitation of cuprous oxide was carried out by the official method of Munson and Walker. The estimation of cuprous oxide was made by the volumetric method described by Bisson and Sewell (2).

PRESENTATION OF DATA

Chloride Content of Normal Jersey Milk

Graphs in Figure 1 show the chloride content of milk samples obtained weekly during a complete lactation period of each of ten animals. Two lactations are shown for animal 7. The milk of animals 1 through 7 (lactation 1) may be regarded as typical of normal milk of the herd.

Data used in Figure 1 are presented statistically in Table 1. The standard deviations and coefficients of variation indicate a wide variation in the mean chloride content of the milk of an animal during a given lactation. Variation between individuals is shown by a range of from 0.091 per cent to 0.136 per cent in the mean chloride content of the milk of the ten animals.

The average chloride content of normal milk of the herd for each week of lactation is shown in Figure 2. This curve was established by the analysis of 690 samples. The general trend of the chloride during lactation is characterized by an initially high value which falls during the first four weeks, then rises gradually until about the 38th week when the rate of increase becomes more pronounced. This rapid rise continues until the end of lactation.

Statistical constants for the mean chloride content of normal milk of the herd are given in Table 2 for 12 four-week periods. No values are included for samples criticized either as "salty" or "rancid." The standard errors set down in Table 2 show the reliability which may be attributed to the calculated means. The degree of dispersion of the chloride of the milk of different animals in the same period of lactation is high and is approximately the same as the variation in the chloride of the milk of the individual animals during lactation.

Chloride Content of Rancid Milk

The production of rancid samples by the animals shown in Figure 1 is typical of the herd. Some of the cows produced no rancid milk; others gave

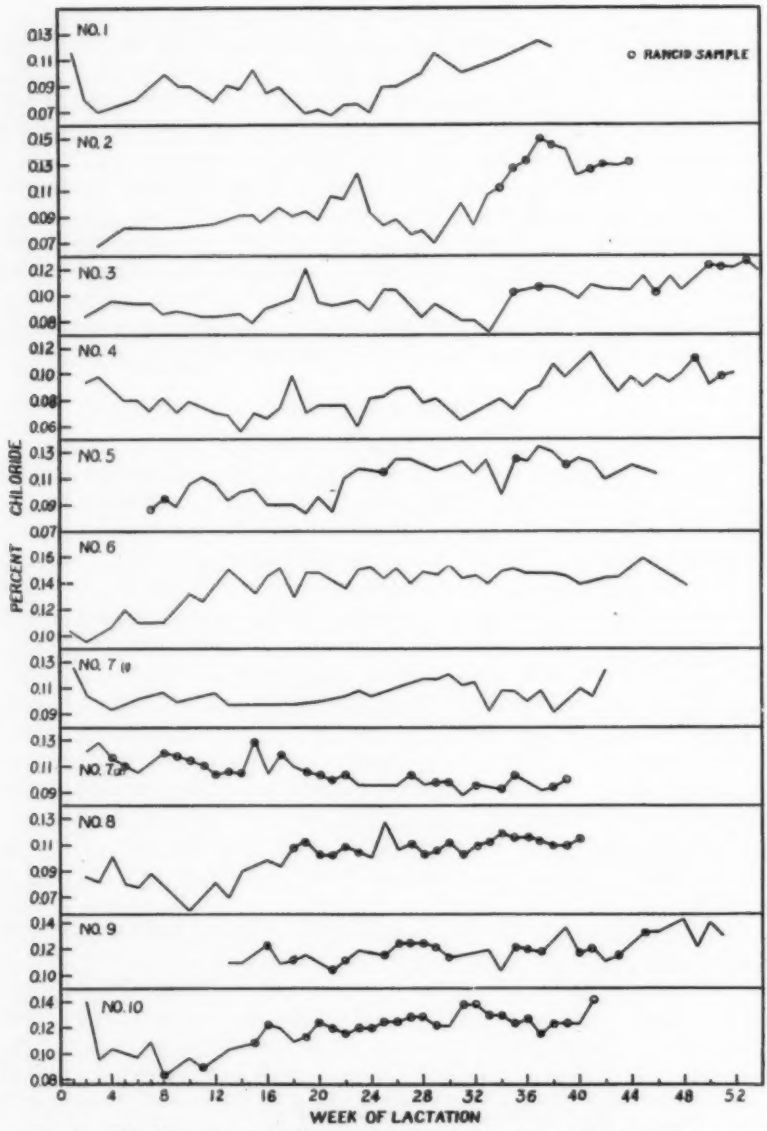

FIG. 1. Chloride content of milk samples taken weekly from each of ten Jersey cows during a complete lactation. Rancid samples are indicated by circles.

TABLE 1

Chloride and lactose content of milk produced by ten[1] Jersey cows during a complete lactation

Cow number	Age	Milk samples	Chloride content			Milk samples	Lactose content		
			Mean chloride	Standard deviation	Coefficients of variation		Mean lactose	Standard deviation	Coefficients of variation
	years	*number*	*per cent*	*per cent*	*per cent*	*number*	*per cent*	*per cent*	*per cent*
1	10	37	0.091 ± 0.003[5]	0.016	17.3	36	4.97 ± 0.04[5]	0.27	5.4
2	4	38	0.099 ± 0.004	0.022	22.1	41	4.78 ± 0.05	0.32	6.6
3	4	52	0.100 ± 0.002	0.014	14.0	44	4.89 ± 0.04	0.23	4.7
4	3	67	0.089 ± 0.002	0.015	16.3	66	4.93 ± 0.04	0.29	5.9
5	4	40	0.114 ± 0.003	0.022	18.9	38	4.89 ± 0.05	0.29	4.6
6	12	48[2]	0.136 ± 0.002	0.016	12.0	45[2]	4.28 ± 0.03	0.18	4.1
7 (1)	11	36	0.105 ± 0.002	0.011	9.9	36	4.66 ± 0.06	0.37	7.8
7 (2)	12	36[3,4]	0.107 ± 0.002	0.010	9.4	29[3,4]	4.93 ± 0.04	0.23	7.8
8	3	39[4]	0.101 ± 0.002	0.012	11.7	34[4]	4.73 ± 0.05	0.29	6.1
9	12	43[4]	0.127 ± 0.002	0.015	11.7	39[4]	4.12 ± 0.04	0.27	6.7
10	7	39[4]	0.117 ± 0.002	0.015	12.7	39[4]	4.67 ± 0.05	0.28	6.0

[1] Two lactations are shown for cow number 7.

[2] Milk criticized as salty during entire lactation.

[3] Chloride decreased, lactose increased with advancing lactation.

[4] More than 50 per cent of the samples were criticized as "rancid."

[5] Standard error of mean.

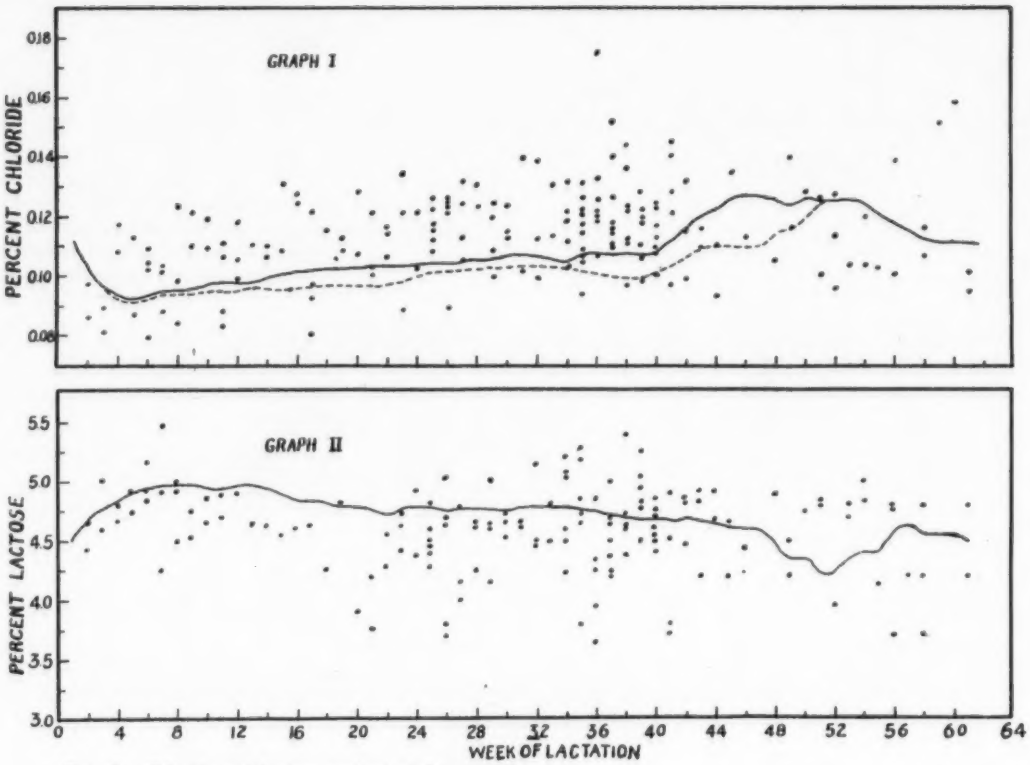

FIG. 2. Chloride and lactose content of Jersey milk.

Graph I —— Mean chloride content of all normal and salty samples for each week of the lactation period.
---- Mean chloride content of all normal samples.
• Chloride content of rancid samples.

Graph II —— Mean lactose content of all normal samples for each week of the lactation period.
• Lactose content of rancid samples.

TABLE 2

Chloride and lactose content of normal[1] Jersey milk in relation to the period of lactation

Lactation period	Milk samples	Chloride content			Milk samples	Lactose content		
		Mean chloride	Standard deviation	Coefficients of variation		Mean lactose	Standard deviation	Coefficients of variation
weeks	*number*	*per cent*	*per cent*	*per cent*	*number*	*per cent*	*per cent*	*per cent*
1– 4	65	0.097 ± 0.002[2]	0.016	16.7	63	4.75 ± 0.043[2]	0.34	7.3
5– 8	67	0.094 ± 0.002	0.013	13.6	63	4.93 ± 0.032	0.25	5.1
9–12	50	0.095 ± 0.002	0.011	12.1	49	4.94 ± 0.048	0.34	6.8
13–16	61	0.095 ± 0.002	0.012	13.0	58	4.85 ± 0.051	0.39	8.1
17–20	43	0.097 ± 0.002	0.014	9.7	45	4.80 ± 0.070	0.47	9.8
21–24	50	0.097 ± 0.002	0.014	14.6	50	4.74 ± 0.048	0.34	7.1
25–28	59	0.100 ± 0.002	0.015	14.9	62	4.81 ± 0.047	0.37	7.7
29–32	62	0.102 ± 0.003	0.021	20.7	65	4.78 ± 0.058	0.46	9.7
33–36	47	0.103 ± 0.003	0.018	17.1	52	4.78 ± 0.059	0.43	8.9
37–40	29	0.103 ± 0.004	0.020	19.0	36	4.69 ± 0.062	0.37	7.9
41–44	23	0.108 ± 0.003	0.014	12.6	30	4.62 ± 0.068	0.37	8.1
45–48	13	0.108 ± 0.003	0.010	9.4	20	4.61 ± 0.042	0.42	9.2

[1] Samples criticized as "salty" or "rancid" are not included.

[2] Standard error of mean.

a few such samples, usually toward the end of lactation; while others produced rancid milk as early as the 4th and 8th weeks of lactation and continued to produce it throughout the remainder of the lactation period.

As may be seen from Figure 1 and Table 1, the chloride of the milk of animals most frequently producing rancid samples was usually higher for a greater part of the lactation period than it was for animals which produced few or no rancid samples. For example, the milk of animals 8, 9 and 10, frequently rancid, showed a consistently high chloride content after the 16th week of lactation, the values ranging from 0.110 per cent to 0.140 per cent. However, during the weeks in which these cows produced normal milk the chloride showed no change. That the high chloride may be characteristic of all the milk of the animal and not of the rancid samples alone is also indicated in the two lactation periods of animal 7. The chloride content of the milk was high in both lactations, although no rancidity occurred until the second lactation.

In order to facilitate a comparison of the chloride content of rancid milk with that of normal milk produced at the same stage of lactation, the chloride content of 179 rancid samples is shown as individual points in Figure 2, in conjunction with the curve of the average chloride content of normal milk. It is evident from the figure that the chloride of the rancid samples was, in most instances, higher than the average chloride of normal milk produced at the same stage of lactation.

If the period of lactation is disregarded, a further comparison of the chloride of normal and rancid milk may be made by grouping the samples as shown in Table 3. The mean chloride content of all rancid samples was 0.1139 ± 0.0012 per cent, that of the normal samples, exclusive of salty samples, 0.0983 ± 0.0008 per cent. The difference between the means of the two groups, 0.0157 per cent, is 11 times the standard error of the difference, 0.0014, and is, therefore, statistically significant.

In considering the chloride of milk, it is of interest to observe the chloride content of samples which were judged "salty." Of 530 samples which had a chloride content of from 0.050 per cent to 0.120 per cent, 25 were criticized as salty, 15 of the "salty" samples containing from 0.101 per cent to 0.120 per cent chloride; of 82 samples containing 0.121 per cent to 0.140 per cent chloride, 34 were considered salty; while in 48 out of 51 samples containing more than 0.141 per cent chloride the salty taste was perceptible.

Lactose Content of Normal Jersey Milk

The graphs in Figure 3 show the changes occurring in the lactose content of the milk of individual animals with the advance of lactation. Variation between different animals in the lactose content of the milk may also be observed for all stages of the lactation period. The lactose content of the milk of animals 1 through 7 (lactation 1) may be regarded as typical for the

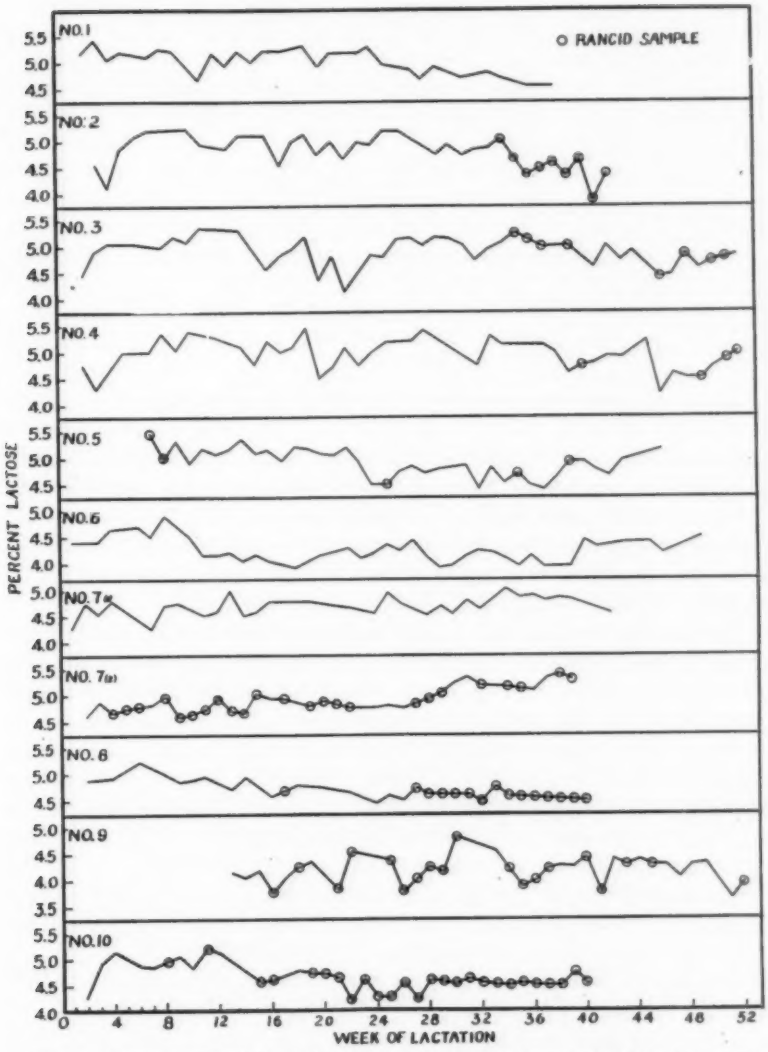

FIG. 3. Lactose content of milk samples taken weekly from each of ten Jersey cows during a complete lactation. Rancid samples are indicated by circles.

TABLE 3

Chloride content of rancid, salty and normal Jersey milk

Description of samples	Milk samples	Chloride content			
		Mean	Standard error	Standard deviations	Coefficients of variation
	number				
"Rancid"[1]	179	0.114	0.001	0.017	14.6
"Salty"	106	0.134	0.002	0.023	16.9
Normal	584	0.098	0.001	0.018	18.5
All samples	869	0.106	0.001	0.022	20.9

[1] The formula used in determining the significance of the difference between the means of the chloride content of normal and "rancid" samples was:

$\frac{M_R - M_N}{\sigma_d}$ in which

M_R = mean chloride of "rancid" samples,
M_N = mean chloride of normal samples,
σ_d = standard error of difference = $\sqrt{(\sigma_R)^2 + (\sigma_N)^2}$;
σ_R = standard error of mean chloride of "rancid" samples,
σ_N = standard error of mean chloride of normal samples.

herd. In Table 1 are found the mean lactose content of the milk of each of the animals represented in Figure 3, together with statistical constants. The coefficients of variation indicate the small degree of variation in the lactose of the milk of an individual animal in the course of a lactation. Individual differences are shown by a range of from 4.11 per cent to 4.97 per cent in the mean lactose content of the milk of the ten cows shown.

The general trend of the lactose content of milk during the lactation period is shown in Figure 2. The graph in this figure represents the average lactose content of all samples of normal milk for each week of the lactation period and was determined by the analysis of 599 samples of milk. As may be seen from the graph, there occurs a slight increase in the lactose content of milk during the first 12 weeks of lactation, followed by a decrease during the succeeding 12-week period, after which the lactose remains constant until a very gradual decrease sets in about the 36th week. The maximum change occurring in the average weekly values was 0.5 per cent.

The above data have been summarized in Table 2 which gives the statistical constants for mean lactose concentrations of all normal samples for each of 12 four-week periods. A comparison of the coefficients of variation for the different periods of lactation with those of individual animals (Table 1) indicates a slightly greater variation in the lactose of the milk of different animals in the same week of lactation than in the lactose of the milk of an individual animal during a complete lactation.

Lactose Content of Rancid Jersey Milk

The lactose content of naturally rancid samples observed during complete lactations of ten cows is indicated on the individual graphs in Figure 3.

A comparison of the lactose of the normal and rancid milk produced by the same animal shows no difference for samples produced at comparable stages of lactation. The level of the lactose content of the milk of animals frequently producing rancid samples was, however, appreciably lower than that of animals whose milk was very seldom rancid, averaging 4.51 per cent for the frequently rancid milk of cows 8, 9 and 10, and 4.87 per cent for the milk of animals 1 through 5, and 7 (lactation 1), which rarely produced rancid samples.

For the purpose of comparison with the lactose of normal milk in the same period of lactation the lactose content of 154 rancid samples is shown as separate points in Figure 2, in conjunction with the graph showing the average lactose content of the milk of the herd. It is evident from the figure that in most cases the lactose content of the rancid samples was less than the average lactose content of normal milk of the same stage of lactation.

The mean lactose content of 599 normal samples irrespective of the period of lactation was 4.79 ± 0.0160 per cent; the standard deviation of the mean ± 0.3914; the coefficient of variation, 8.17. The mean lactose percentage of 138 rancid samples was 4.62 ± 0.0318 per cent; the standard deviation of the mean ± 0.3733; the percentage coefficient of variation, 8.09.

The mean lactose of rancid samples was significantly lower than the mean lactose of normal samples, the difference between them, 0.17 per cent, being 4.8 times the standard error of the difference, ± 0.0357. The lower lactose content of the rancid samples is attributed to the fact that animals whose milk is frequently rancid produce milk having a consistently lower lactose content than do animals whose milk is seldom rancid.

Chloride-Lactose Ratio of Normal and Rancid Jersey Milk

Roadhouse and Koestler (13) regard the chloride-lactose relation as one of the most important bases of milk taste. They have found that milk samples with a high chloride-lactose ratio are judged less favorably than those of like origin where the ratio is relatively low.

In the present experiment the average chloride-lactose ratio of normal milk was found to remain almost constant throughout the greater part of the lactation period except for an initial drop from 2.6 to 1.9 in the first four weeks of lactation, and a rise from 2.25 to 2.75 after the 42nd week. From the 5th to the 42nd week the ratio showed only a slight increase of from 1.9 to 2.25.

The chloride-lactose ratio of the rancid samples was in most cases higher than the average ratio for normal milk of a comparable period of lactation; fifty per cent of the rancid samples had a chloride-lactose ratio lying between 2.5 and 3.5 when the ratio of the normal samples lay between 1.9 and 2.25. The higher ratio of the rancid samples is the result of the increased chloride and decreased lactose of the rancid samples.

SUMMARY AND CONCLUSIONS

Data have been presented showing the chloride and lactose content of the milk of animals of a Jersey herd, all of which received the same ration and were exposed to the same environmental conditions. The amounts of these constituents found in milk criticized as rancid have been compared with the amounts present in normal milk produced during the same period of lactation. The data are presented statistically and graphically both for individual animals and for the herd.

Milk from cows frequently producing rancid samples has a higher chloride and a lower lactose content than normal milk produced in the same period of lactation. The chloride-lactose ratio of rancid milk is high. The high chloride and low lactose content appears to be characteristic of all milk produced by those animals whose milk is frequently rancid.

Occasional rancidity occurring in the milk of animals usually producing normal milk, or conversely, the production of normal milk by animals frequently producing rancid samples, cannot be explained on the basis of changes in the chloride and lactose content of the milk.

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EFFECT OF pH ON THE PRODUCTION OF ACETYLMETHYLCARBINOL PLUS DIACETYL IN MILK BY THE CITRIC ACID FERMENTING STREPTOCOCCI

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Although the acetylmethylcarbinol plus diacetyl content of milk cultures of the citric acid fermenting streptococci (*Streptococcus citrovorus* and *Streptococcus paracitrovorus*) is normally very low, the adjustment of the pH to the proper point after the bacterial population has increased extensively results in a rapid accumulation of these compounds. Various acids can be used for this purpose (1). Since the organisms reduce acetylmethylcarbinol and diacetyl under favorable growth conditions (2), they may produce the compounds in the absence of added acid and then quickly reduce them while with added acid the reduction is delayed by the unsatisfactory conditions. In a butter culture a pH favorable for the accumulation of acetylmethylcarbinol and diacetyl is established through the production of lactic acid by *Streptococcus lactis* which is present along with the citric acid fermenting organisms.

The work herein reported shows the relationship of different pH values, as produced by various acids, to the production of acetylmethylcarbinol plus diacetyl in milk cultures of the citric acid fermenting streptococci.

PROCEDURE AND METHODS

For each trial, 300 ml. portions of skimmilk were added to pint milk bottles and sterilized in an autoclave. After the milk had cooled to 21° C., the bottles were inoculated with equal amounts of a milk culture of the organism to be studied and incubated about 24 to 40 hours at 21° C. to permit the development of a large number of bacterial cells. The various cultures were then adjusted to the desired pH values with the different acids, and again incubated for about 48 hours at 21° C. to permit the production of acetylmethylcarbinol plus diacetyl. The acids used were citric, lactic, sulfuric, and 0.15 per cent lactic acid together with the amount of sulphuric necessary to yield the desired pH; the acids were made up as aqueous solutions and sterilized. The acetylmethylcarbinol plus diacetyl in the cultures was determined as nickel dimethylglyoximate per 200 gm. of culture (3). Only one incubation period was used and it is probable that the maximum yield of the carbinol plus diacetyl was not obtained since the content of these materials is not constant in a culture and is either increasing or decreasing, but the incubation conditions were favorable for a high yield and accordingly the results are satisfactory for comparative purposes.

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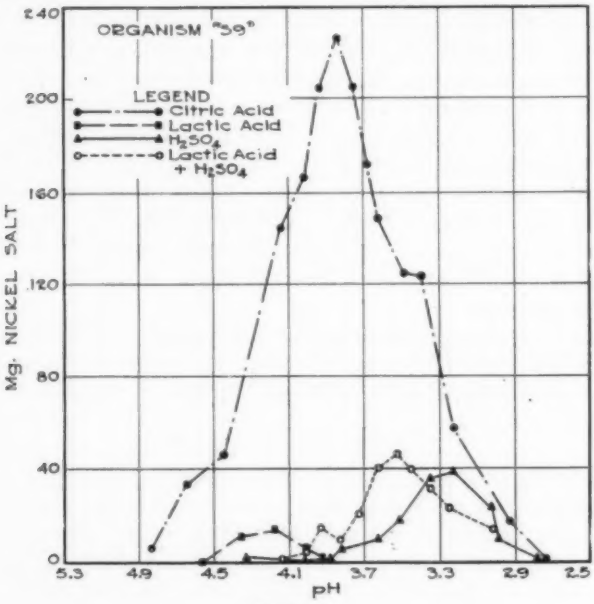
The pH determinations were made electrometrically with a quinhydrone electrode as soon as the acid was added. During the production of carbinol plus diacetyl in a culture the pH commonly increases (1).

RESULTS OBTAINED

The data are presented in the form of graphs, each graph giving the results of one trial.

Graph 1 shows data on organism S9. The importance of citric acid as the

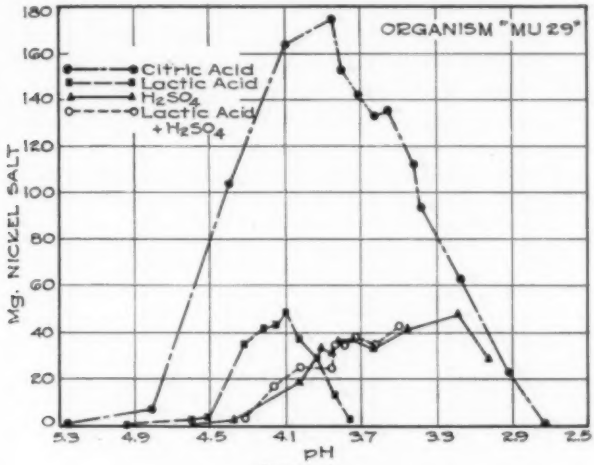


GRAPH 1

source of acetylmethylcarbinol plus diacetyl is evident from the relatively high yields resulting when it was added to the cultures. The maximum yield was obtained at a pH of 3.8.[1] This acid gave a comparatively high yield of the carbinol plus diacetyl over a wide pH range. Lactic acid gave the lowest yields, with the maximum at pH 4.2, and a significant production occurred over a rather narrow pH range. Sulphuric acid gave higher yields than lactic acid and the maximum yield was at pH 3.2; with it the pH range yielding a significant production was wider than with lactic acid. The mixture of lactic and sulphuric acids resembled sulphuric acid alone in the maximum yield of acetylmethycarbinol plus diacetyl and in the width of the pH range giving a significant production, but the pH at which the maximum yield occurred was 3.5.

[1] In discussing pH the values given are to the nearest 0.1.

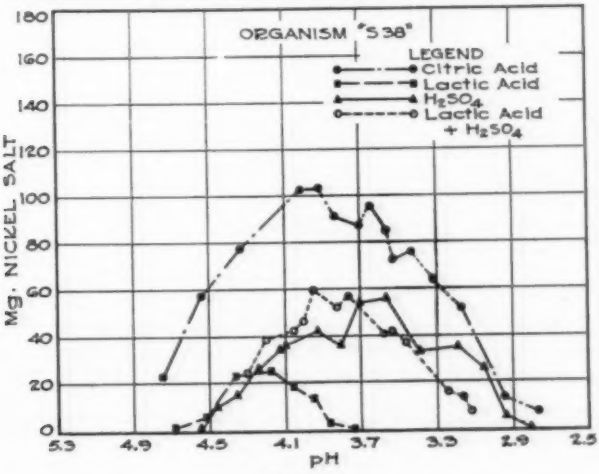
Results on organism MU29 are presented in Graph 2. Citric acid gave a



GRAPH 2

high yield of acetylmethylcarbinol plus diacetyl over a wide pH range with a maximum at pH 3.8. Lactic acid gave a higher maximum yield than with organism S9, the maximum being essentially the same as with sulphuric acid. However, with lactic acid the maximum yield was at a higher pH (4.1) than with sulfuric acid (3.2) and a significant production occurred over a narrower pH range.

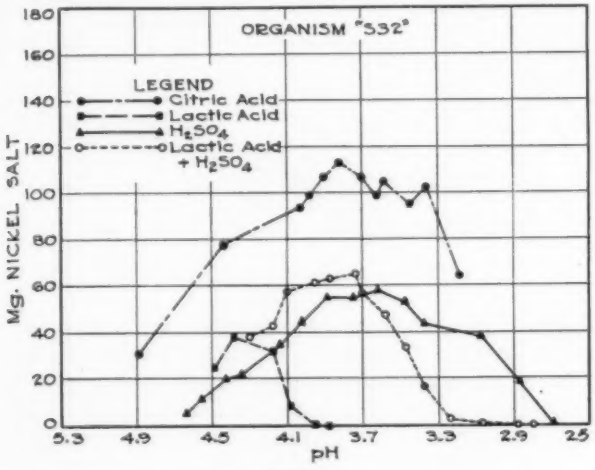
Data on organism S38 are given in Graph 3. With citric acid the maxi-



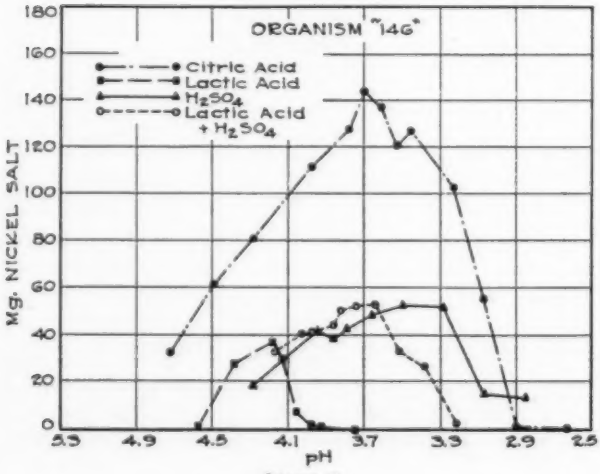
GRAPH 3

mum yield of acetylmethylcarbinol plus diacetyl, which occurred at a pH of 3.9, was not as high as with the organisms already considered but a significant production was obtained over a wide pH range. Lactic acid gave a relatively low production over a narrow pH range, with the maximum yield at pH 4.2, while sulphuric acid gave a somewhat higher production over a wider range, the maximum being at pH 3.6. The mixture of lactic and sulphuric acids gave a maximum yield at a pH of 3.9.

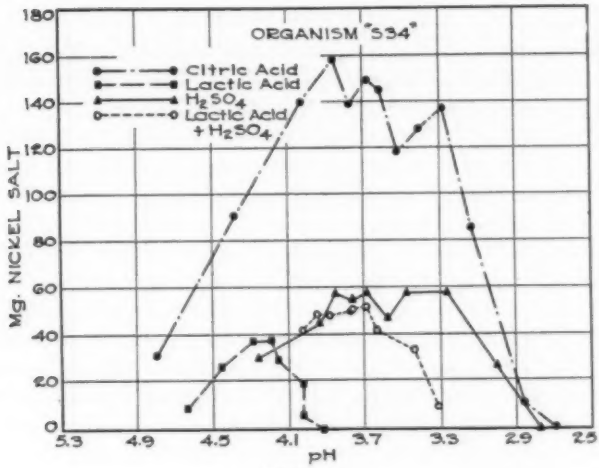
Graphs 4, 5, 6, 7 present data on organisms S32, 146, S34, and DAN2,



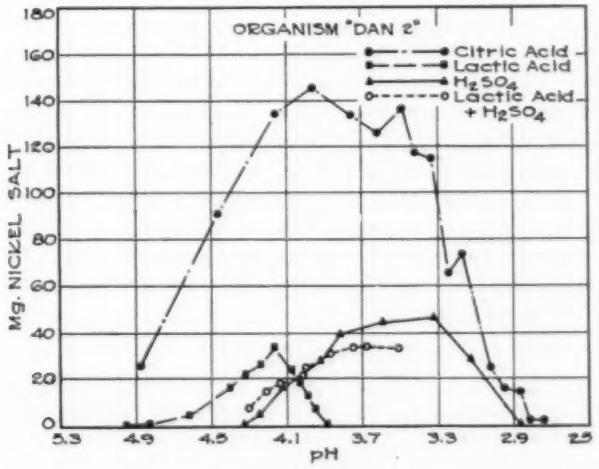
GRAPH 4



GRAPH 5



GRAPH 6



GRAPH 7

respectively. Each of these organisms gave essentially the same results as organism S38. Organism S32 gave the maximum yields of acetylmethylcarbinol plus diacetyl with citric, lactic, sulphuric, and the mixed acids at pH values of 3.8, 4.4, 3.6, and 3.7, respectively; organism 146 gave them at pH values of 3.7, 4.2, 3.5, and 3.6, respectively; organism S34 gave them at pH values of 3.8, 4.2, 3.2, and 3.7, respectively; and organism DAN2 gave them at pH values of 3.9, 4.2, 3.3, and 3.7, respectively.

SUMMARY AND DISCUSSION

The graphs presented, which represent data obtained on seven cultures of the citric acid fermenting streptococci, emphasize the production of acetylmethylcarbinol plus diacetyl from citric acid. When this acid was used to acidify milk cultures of the organisms, the yield of the carbinol plus diacetyl was always much higher than when other acids were used and a significant production occurred over a wide pH range; the maximum yields were obtained at pH values from 3.9 to 3.7.

With lactic acid as the acidulant, comparatively low yields of acetylmethylcarbinol plus diacetyl were obtained and a significant production occurred over a rather narrow pH range. The maximum yields were obtained at pH values from 4.4 to 4.1 which approximate the pH values commonly found in satisfactory butter cultures. Since lactic acid is the principal acid involved in establishing the pH in butter cultures, the frequent failure to obtain a high yield of acetylmethylcarbinol plus diacetyl in such cultures may be due to the relatively unfavorable character of lactic acid. Sulphuric acid gave higher yields of acetylmethylcarbinol plus diacetyl than lactic acid and also gave a significant production over a wider pH range. The maximum yields occurred at comparatively low pH values which ranged from 3.6 to 3.2. The favorable character of sulphuric acid compared to lactic acid for the production of acetylmethylcarbinol plus diacetyl may explain why the special butter culture made by growing one of the citric acid fermenting streptococci in milk and then acidifying with citric and sulphuric acids (1) are so generally satisfactory. A mixture of 0.15 per cent lactic and variable amounts of sulphuric acid, depending on the pH desired, gave yields of acetylmethylcarbinol plus diacetyl comparable to those obtained with sulphuric acid alone. The maximum yields occurred at pH values from 3.9 to 3.5. In general, these values were between the corresponding values for lactic and sulphuric acids.

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FLAVOR AND BACTERIAL CHANGES OCCURRING DURING STORAGE OF SWEET CREAM WHICH HAS BEEN FLASH PASTEURIZED AT VARIOUS TEMPERATURES

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The problem of maintaining a desirable flavor in high quality sweet cream involves the control of the bacterial flora in the cream. Since the temperature at which the cream is heated would influence the flora it seemed desirable to study the bacteria which survived the flash pasteurization process. It was hoped that a relationship might be found between the surviving organisms and the final flavor of the cream. The work was undertaken initially as a plant control measure and is offered to others who are interested in similar problems.

Numerous investigators have studied the bacteria which survive in milk pasteurized by the holding method, but very few studies have come to the attention of the author on the high-temperature, short-time or flash process of heating. The work published on the latter method has dealt mainly with thermophilic bacteria (1) in milk or has been limited to other products such as butter. Many of the previous investigations have included studies of the physiologic types of organisms surviving in milk heated for 30 minutes at various temperatures. The method used by Ayers and Johnson (2) to differentiate the various types was to pick individual colonies of bacteria from agar plates and inoculate separate tubes of sterile litmus milk with one colony each; the tubes were incubated for a period of 14 days at 30° C. and the reactions then observed. These investigators divided the organisms into the following groups: acid forming and coagulating, acid forming, inert, alkali forming and peptonizing. Two additional groups, namely, acid coagulating-peptonizing and sweet curdling were added to the above list by Thurston and Olson (3) who employed the "milk-tube" method of Ayers and Johnson in an investigation of high grade milk before and after pasteurization.

In an effort to secure maximum growth of colonies on standard agar, Thurston and Olson incubated their plates 3 days at 19°–21° C., then 2 days at 37° C., before picking the colonies into sterile litmus milk. Several proposals have been advanced to alter the media used in the bacteriological analysis of milk in order to secure more suitable conditions for growth of milk bacteria. The tryptone-glucose-skimmilk agar suggested by Bowers and Hucker (4) was used throughout this study.

Different temperatures of incubation for the agar plates likewise have

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been proposed by others but in this study limited incubator capacity permitted only the use of 37° C. for 48 hours.

PROCEDURE

The raw milks which were separated to supply the creams used throughout this investigation were obtained from the daily plant receipts and were representative of approximately twenty thousand pound lots. Samples of the milk were collected in sterile vials at intervals during the separation of the experimental cream and were mixed just previous to plating. About 25 pounds of raw cream testing 44 to 46 per cent fat were obtained directly from the separators and were taken to the laboratory for immediate processing. However, in two experiments the cream was processed in the large barrel-type commercial flash pasteurizer used in the daily plant operation.

Six 100 ml. samples of the raw cream were taken in sterile, cotton-stoppered flasks and placed immediately in ice water. The remainder of the cream was processed by flash pasteurization to 185°, 175°, 165° or 155° F. in the laboratory apparatus previously described by the author (5). The pasteurizer consists of 1-, 1.5- and 2-inch diameter tinned-copper sanitary piping which is telescoped and is fitted with appropriate inlets and outlets for the heating water and the cream. The cream flows through the intermediate section and is heated with hot water which is pumped through the center and outside sections. The temperature of the heating medium was 10° to 15° F. higher than the final temperature of the cream, the latter requiring from 30 to 45 seconds to pass through the pasteurizer. The hot cream was immediately directed over a small tinned-copper surface cooler and was cooled at once to 50° F. Approximately five seconds were required for the cream to flow from the pasteurizer to the surface cooler. The cream heated to 185° F. was pasteurized first and the temperature progressively lowered with adequate time elapsing between collection of the samples so that thorough flushing of the equipment with the cooler cream was assured. Six sterile, cotton-stoppered flasks were filled with the cooled cream from each lot and immersed at once in ice water. All pieces of equipment used in handling the cream were previously scalded for five minutes with boiling water.

The sample flasks of cream were held in the ice-water bath for three hours and were then transferred to a refrigerator maintained at 35° F. One flask of cream from each lot was retained for bacterial and flavor analyses and the procedure repeated every two days thereafter, the final test usually being made when the cream was ten days old. The bacteriological methods followed were the standard procedure (6) with the substitution of tryptone-glucose-skimmilk agar for the standard nutrient media. Duplicate plates were always made for each lot of cream and the dilutions that were necessary to secure plates containing from 30 to 60 colonies were 1:10 and 1:100 for the heated creams while the raw creams occasionally required 1:1000. Fre-

quently the number of colonies found on the plates were considerably above or below the desired limits. All of the plates were incubated at 37° C. for 48 hours before they were counted.

After the samples had been plated the cream remaining in the flasks was warmed to 90° F. and used for flavor analysis; the criticisms were made by two judges.

Immediately after the plates were counted the individual colonies were picked off with a sterile needle and each colony was placed in a separate tube of sterile litmus milk. An effort was made to get from 30 to 60 colonies from each sample but the plates made with the cream which had been pasteurized at 175° and 185° F. usually did not contain that many colonies; in this case all colonies were taken from the two plates. When more than 60 colonies grew on a plate an area containing about that number was chosen and all colonies in that area were picked; selective picking was thus avoided. The inoculated tubes were incubated at 30° C. for 10 days before the final observations were made. The bacteria were divided according to the reactions produced in the litmus milk into the following groups: inert, alkali forming, acid forming, acid coagulating, acid coagulating-peptonizing and sweet curdling.

Incubated tubes of litmus milk frequently were selected from each grouping of bacteria and smears prepared and examined microscopically; the morphologic class of the various organisms was thus determined.

RESULTS

Bacterial Changes

Twelve experiments were completed during the months from November until the following July, so that milks produced during the winter, spring and summer months were included in the study. In Table 1 are presented

TABLE 1

Number of bacteria in sweet cream flash pasteurized at various temperatures and stored at 35° F. for several days

Temperature of flash pasteurization	Number of bacteria per ml. after storage—days					
	0	2	4	6	8	10
°F.						
Orig. Milk	17,950					
Raw Cream	9,296	10,740	15,560	20,140	31,850	97,950
155	1,146	1,217	1,453	1,135	1,242	1,775
165	429	374	461	407	392	495
175	171	188	233	137	185	167
185	115	139	104	98	123	104

the bacterial counts of the flash pasteurized creams during the ten day storage period at 35° F. These counts are averages of the twelve experiments and

were computed logarithmetically. The average count of the original raw milks is also included and all of these counts were below 20,000 except one sample each obtained in February, May and July where the counts were 35,000, 40,000 and 73,000. The counts of the raw creams produced from these milks were likewise higher than the others. In the group of pasteurized creams the counts for each range of temperature were quite uniform in all of the experiments although the creams heated at 155° and 165° F. varied more than those heated at 175° and 185° F.

It is evident from the results that the organisms which survive decrease as the heating temperature of the cream is increased. Since more organisms are present in the raw creams than survive in the heated portions the increases in the former, during the storage period, are proportionately greater. The growth curve of the organisms in the raw creams during storage is decidedly upward whereas those of the heated creams are comparatively flat in shape.

The changing picture of bacterial life in the creams is more vividly portrayed in Fig. 1. This chart shows the average percentages of the various

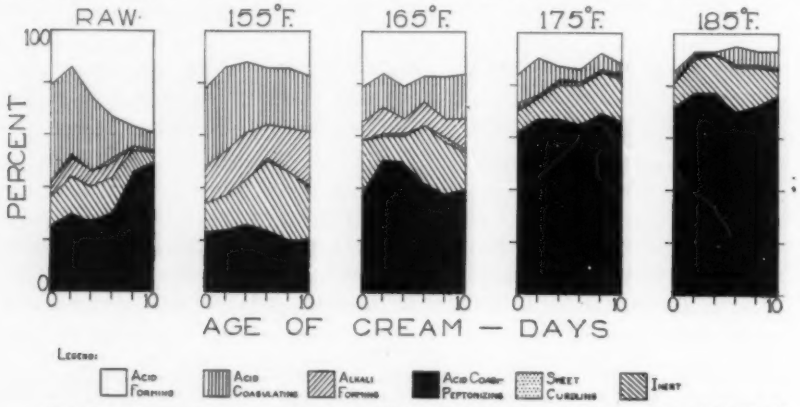

FIG. 1. Changes in the percentage of the various physiologic groups of bacteria which occur in raw and pasteurized sweet cream during storage at 35° F.

physiologic types of organisms surviving in the creams throughout the ten-day storage period and was constructed with the data resulting from the study of the inoculated tubes of litmus milk. In the fresh raw creams the acid coagulating organisms are the largest group, but as these creams are held in storage for ten days at 35° F. the number decreases, particularly after the second day. The acid coagulating-peptonizing and the acid forming groups increase during storage so that after eight days the peptonizers predominate all other groups, although the acid formers are nearly as prevalent.

In the pasteurized creams the acid coagulating group of bacteria are still the largest in those samples heated at 155° F. but do not tend to decrease as

rapidly during storage as in the raw creams. The peptonizing bacteria are reduced as the result of the heat-treatment at 155° F. and do not increase during the storage period. Also a rather large number of alkali forming organisms exists throughout the storage period in the creams heated at the lower temperature. As the heating temperature is raised the percentage of peptonizing bacteria likewise increased; at 155° F. about 25 per cent of the organisms were peptonizers whereas they increased to approximately 40 per cent at 165°, 60 per cent at 175° and 75 per cent at 185° F. This increase of peptonizers was accompanied by decreases in the other groups of organisms which were destroyed by the heat-treatment; the alkali forming group nearly disappeared at 175° F. and above while the acid forming and acid coagulating groups progressively decreased as the heating temperature rose.

The group of inert organisms produce no change in litmus milk; they are present in all samples of cream but represent, mainly, tubes of litmus milk in which the inoculated bacteria failed to grow. It is felt that this group is not an accurate picture of the truly inert organisms, for they were very few in number. If the tubes in which no growth of organisms occurred had produced a growth the final percentages of the other groups would have been altered considerably. Although a few of the acid coagulating group exist at 175° and 185° F. it appeared that a considerable portion of these would peptonize if the tubes were incubated several days more.

The morphologic groups of bacteria which produced the various reactions in litmus milk occasionally were determined microscopically and the observations are presented in Table 2. The table indicates that the acid coagulating

TABLE 2

Morphologic classes of bacteria from raw and flash pasteurized creams which produced various reactions in litmus milk

Type of reaction	Classification of bacteria observed
Acid Coagulating	Streptococci—paired and short-chained
Acid Forming	Cocci and bacilli
Acid Coagulating—Peptonizing	Bacilli and cocci
Alkali Forming	Bacilli
Sweet Curdling	Bacilli

organisms were paired or short chained streptococci whereas those which only formed acid were cocci and bacilli. The groups responsible for the acid coagulating-peptonizing type of reactions were likewise bacilli and cocci organisms; the former group also was responsible for the alkali forming and sweet curdling reactions. The temperature at which the cream was pasteurized did not seem to influence the morphologic class of the bacteria which produced a certain type of reaction. An example of this is shown by the acid coagulating-peptonizing bacilli and cocci which were observed in the litmus milk tubes inoculated with colonies from raw cream and from creams flash pasteurized at each temperature studied.

Flavor Changes

Associated with the study of the bacterial changes in the cream during storage a parallel study was made of the changes in the predominating flavors of the cream and the results are summarized in Table 3. This table reveals

TABLE 3

Changes in the flavor of flash pasteurized sweet cream during storage for several days at 55° F.

Temperature of flash pasteurization	Flavor of cream after storage—days					
	0	2	4	6	8	10
°F.						
Raw Cream	Good	Slightly Bitter	Bitter	Bitter	Very Bitter	Very Bitter
155	Fine	Very Good	Good	Good	Slightly Old	Old, Stale
165	Slightly Heated	Fine	Fine	Very Good	Good	Good
175	Heated	Heated	Slightly Heated	Slightly Heated	Slightly Heated	Slightly Heated
185	Pronounced Heated	Pronounced Heated	Pronounced Heated	Heated	Heated	Heated

that very undesirable changes in flavor occur in the raw creams soon after separation from the raw milk; after two days storage all of the raw samples, except the two obtained in the summer months, were bitter. The raw cream produced the last week in May retained a fine flavor for six days while that produced the last week in July was slightly stale on the fourth day. The changing seasons of the year accompanied with the changes in the feeds which the cows consume is reflected in the flavors and the ability of the creams to maintain the desirable ones.

The effect of flash pasteurization is to retard these undesirable changes and to prolong the keeping quality of the stored product. A temperature is reached, however, where a heated taste is imparted to the cream, for flash pasteurization at temperatures above 165° F. produced objectionable heated flavors. During the winter and early spring months a pasteurizing temperature of 165° F. generally resulted in producing a cream of the most desirable flavor, whereas the cream produced in the late spring and summer months retained its fine flavors when heated to 155° F. When the cows were on fresh grass pasture in the spring a grassy flavor was imparted to the cream, pasteurization tending to amplify it so that at 165° the flavor was more pronounced than when the heating was limited to 155° F. Creams heated at 175° F. and above usually possessed such a heated flavor that other distinctions were impossible.

Flavor changes occurring between 155° F. and 165° F. in fresh cream are difficult to describe since they are delicate ones to detect. A rich, full, fresh cream flavor is evident when the cream is flash pasteurized at 155° F. whereas at 165° F. the delicate blending of flavors disappears and a harshness or a sharp flavor arises. The higher pasteurization also causes the creams to taste sweeter as though a small amount of sugar, such as lactose, has been added. This apparent increased sweetness is replaced by a sharper heated flavor when the creams are pasteurized at 175° F. and above. Some of the heated flavor detectable in the fresh pasteurized creams disappears upon storage so that many of the samples which had objectionable heated flavors when fresh were classed as very good flavored creams when two to four days old. Creams produced in the late spring and early summer have a more desirable flavor than those produced during the winter months.

Since the study involved numerous separate experiments in which the creams were produced during different seasons of the year it appears that other biological factors in addition to the bacteria no doubt have an important bearing on the flavors of the cream. It is possible that these factors may have been elaborated by the bacteria, but the development of that phase of the problem was beyond the scope of this immediate study.

CONCLUSIONS

1. The number of bacteria surviving in sweet cream decreases after flash pasteurization; the reduction occurs inversely to the temperature of heating.

2. The growth of organisms during storage at 35° F. is more rapid in raw than in pasteurized creams. The growth curves in the latter are nearly flat during the ten day storage.

3. In the fresh raw cream the acid coagulating group of bacteria predominates but is gradually replaced by the slower growing peptonizing and the acid forming types as the cream ages.

4. In the flash pasteurized creams the percentages of peptonizing bacteria which survive increases as the heating temperature is raised; this type predominates in the creams heated at 165° F. and above.

5. It is difficult to associate the percentages of the physiologic groups of bacteria present in the various samples with the flavors which occur in the creams; other biological factors evidently play an important part.

6. Very objectionable bitter flavors arise in raw creams shortly after storage.

7. Flash pasteurization at 165° F. prevented the formation of bitter flavors during the 10 day storage period.

8. During the late spring and summer months flash pasteurization at 155° F. produced very fine flavored creams which could be stored for 10 days at 35° F. without objectionable changes in flavor.

9. Flash pasteurization at temperatures above 165° F. imparts objectionable heated flavors to the cream.

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CONCERNING THE ACCURACY OF THE METHYLENE BLUE REDUCTION TEST[1]

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From time to time discussion has centered about the accuracy of the methylene blue reduction test. Various opinions have been expressed, due no doubt to the fact that different workers have held divergent views as to what the test is supposed to measure.

It is generally accepted nowadays that the decolorization of methylene blue follows the removal of dissolved oxygen from the milk (3, 24, 29, 32). With the ordinary grades of raw milk, this oxygen consumption is believed to be due almost entirely to the respiratory activities of bacteria growing in the milk.[2] Since there is, on the average, a fairly satisfactory correlation between bacterial numbers and reduction time (9, 16, 20, 24, 32), the test has been regarded by some as an index of the number of organisms initially present, from which certain deductions may be made regarding the care exercised in production and handling. On the other hand, those interested in the distribution and processing of milk have been further interested in the test as an index of probable keeping quality, a function which it has been found to perform with reasonable satisfaction (5, 16, 24, 32). The improbability that the test will indicate both numbers *and* keeping quality at the same time with perfect accuracy will be evident from the following:

If the test is regarded purely as a measure of bacterial numbers, then complete accuracy would require that all samples of milk containing the same number of organisms should show identical reduction times. That such perfect correlation will rarely be obtained is evident from the following considerations:

1. Since oxygen consumption is proportional to protoplasmic mass (22), larger cells will generally take up oxygen more rapidly than smaller ones.
2. Different species of bacteria show different reducing intensities (6, 15, 32) and different growth rates at 37° C.
3. Bacteria consume oxygen throughout the lag phase of growth (20, 32); thus a given sample will show a shorter reduction time at the end of this phase although the number of bacteria remains the same.

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[2] In the case of milks with few bacteria, it is believed that leucocytes and other body cells, as well as certain milk constituents, may influence reduction time (13, 24). Certain workers (4, 13) have reported that mastitis milk may reduce more rapidly than would be expected on the basis of the bacterial content, and attribute this to leucocytes. Others (18, 19, 25, 32) have failed to confirm this relationship.

4. The bacteriostatic principle (17) is not of equal influence in all milks or on all species of bacteria (15).
5. Cells dormant from prolonged refrigeration may take longer to reach their maximum growth rate than those not so treated (2, 7).
6. Other milk constituents, as well as body cells, may consume oxygen (13, 24) and influence the reduction time where few bacteria are present.
7. The sweeping of varying proportions of bacteria and body cells from the body of the milk by the rising butterfat often results in variable and delayed reduction times (8, 16, 21, 24, 32).

In addition to these factors affecting the reduction time, it must be borne in mind that none of our present methods for counting bacteria shows a high degree of accuracy (14, 23, 31, 32). Most workers, therefore, would agree with the conclusion reached by Hastings (11) in 1919: "It seems evident from the data submitted that the reduction test determines the number of bacteria as accurately as can be done by any method."

On the other hand, if the reduction test is regarded mainly as an indicator of keeping quality, perfect accuracy would demand exact agreement between reduction time and keeping time.[3] That such will rarely be encountered is evident from the following considerations:

1. The rate of formation of by-products which adversely affect the flavor of milk at 15.5° may not be closely related to the rate of oxygen uptake at 37°. Different types of organisms will tend to predominate at the two temperatures (10, 32).
2. Certain important off-flavors which develop in milk on holding (rancid, bitter, oxidized) are not due to bacterial action.
3. Creaming removes bacteria, leucocytes, etc., from the main body of the milk, as previously mentioned.

Satisfactory comparisons between reduction time and keeping time are also hampered by the difficulty of making accurate determinations of the latter, due to the subjective nature of organoleptic tests. Nevertheless, there is fairly general agreement that "the methylene blue reduction test is as accurate a measure of the keeping quality of milk as any method yet available" (24).[4]

From the foregoing, it will be evident that a certain feature of the reduction test may be regarded as advantageous from the one standpoint, but as the reverse from another standpoint. The sensitivity to metabolic activity dur-

[3] The expression "keeping time" refers to the length of time during which a sample held at 15.5° C. will remain sweet and free from off-flavors and odors.

[4] While Robertson and Frayer (20) found the plate count to be slightly more accurate than the reduction test as an indicator of keeping quality in low count milk, Wilson (32), using a modified technic for the reduction test, reports significantly greater accuracy with the latter than with the plate count.

ing the lag phase of growth is an excellent example. From the standpoint of measuring the true bacteriological condition of the milk, this is a decided advantage, particularly with reference to probable keeping quality. On the other hand, from the standpoint of indicating the *number* of bacteria present, this is a disadvantage, and would be considered a source of error. Consequently, it is believed that the reduction test should be regarded, not solely as a measure of bacterial numbers, but rather as an index of the bacteriological *condition* of the milk, since this would include *activity* as well as *numbers*.

Of the various factors listed above which tend to influence the results of the reduction test there is an important one which can readily be eliminated. Various workers have shown that when the fat (and accompanying bacteria and body cells) is prevented from accumulating at the surface by homogenization, addition of agar or rennet (24, 32), or is redistributed at intervals by inversion or shaking (8, 16, 21, 24, 29, 32), decolorization is uniform, reduction time is usually shortened and marked variations between replicate tubes tend to disappear. This is most noticeable with milks containing few bacteria. Wilson (32) has therefore recommended a modified test, the main feature of which is the inversion of the tubes at half-hourly intervals. This has been adopted in England as the official method for the grading of raw milks. Thornton (28, 29), on the other hand, has recently opposed the adoption of this modification on the grounds that (a) the accuracy of the test is not significantly increased thereby, and (b) the technic is complicated unduly. Although the greater variability of standard reduction times of replicate tubes is freely admitted, the question is raised as to whether such variability is an adequate measure of the relative accuracies of the standard and modified reduction tests.[5]

It should be emphasized at this point that Thornton regards the reduction test purely as a measure of initial bacterial content. As such, he considers that the errors inherent in the test are of such magnitude that the significance of variations in standard reduction times of replicates, or differences between standard and modified reduction times, is difficult to evaluate. Even when the test is regarded in the above light it is difficult to accept the view that the errors introduced by creaming in the standard test are not of sufficient importance to warrant their elimination through a slight change in technic. As previously indicated, however, the test is primarily a measure of bacterial activity as indicated by oxygen consumption. The assumption that such a test is inaccurate unless there is perfect agreement between bacterial activity and bacterial *numbers* appears to be unsound and unwarranted. While it is true that certain workers, particularly those engaged

[5] The term "standard reduction test" refers to the test as officially described in the Standard Methods of Milk Analysis, 6th Ed. (1). The term "modified reduction test" indicates that the tubes are inverted at regular (half-hourly or hourly) intervals during incubation.

in official milk control, have tended to regard the reduction time as a quantitative measure of the bacterial content of the milk at the start of incubation, it is believed that the majority would agree with the view expressed by Hastings *et al.* (12) more than 15 years ago: "From many points of view the bacteriologist is chiefly interested in those organisms which are actually growing in milk. It seems probable that the methylene blue reduction test, because it is influenced by these relationships, measures more accurately than does any other method the bacterial activity in the milk."

When the test is regarded as indicating the bacteriological *condition* of the milk, rather than the initial bacterial content, then the basis for Thornton's first objection disappears. As an index of bacterial activity, the modified test undoubtedly possesses a significantly higher degree of accuracy than does the standard test. An accurate determination of the true rate of oxygen consumption is obviously impossible if varying proportions of the bacteria are permitted to concentrate at the surface in the cream layer. Such organisms are unable to affect the oxygen tension of the main body of the milk. The remaining bacteria take longer to consume the oxygen, while there is often irregular decolorization of the dye and poor agreement between replicate tubes. These features virtually disappear when steps are taken to maintain the bacteria in a more uniform state of dispersion. That the small amounts of extra oxygen incorporated in the milk by periodical inversion are of no significance is shown by the excellent agreement between reduction times of homogenized milks by the standard and modified tests (24, 32). It is evident, therefore, that the modified test measures the rate of oxygen consumption with significantly greater accuracy than does the standard test.

In a recent publication (28) Thornton states, "A coefficient of correlation between the results of the standard and modified methylene blue reduction tests calculated for 332 market milks of many classes was found to be 0.94 ± 0.004. Consequently neither test greatly excels the other in accuracy, despite the greater variability displayed in the standard test which may lead to inaccuracy in evaluating an individual milk." The argument advanced here is obviously refuted by the statement in the final clause. If the standard reduction times for individual samples are variable and inaccurate, as Thornton admits, while modified reduction times are not (8, 16, 24, 29, 32) then the two tests cannot be equally accurate. The high coefficient of correlation reported is entirely beside the point, since this is obtained by averaging the results from 332 different milks. It tells absolutely nothing concerning the relative reliability of single tube determinations on *individual* milks, the point in which the analyst is interested. It is therefore difficult to avoid the conclusion that the modified test, with its extremely close agreement between the individual and mean values for a series of replicates, must be regarded as significantly more accurate and reliable than the standard test.

Frayer (8) has recently reported comparative studies with the standard and modified technics using 50 to 100 replicate tubes with each method. His results emphasize the inherent unreliability of the standard method. He points out that if single or duplicate tests had been made on these milks by the standard test it would often have been impossible to predict in which of two or more classes they might have been placed. This point deserves more consideration than it has received heretofore; since in routine testing only single tubes are set up, it is highly important that the method employed yield dependable results. A further illustration of the effect of variations in the standard test upon the classification of milks is afforded by the present

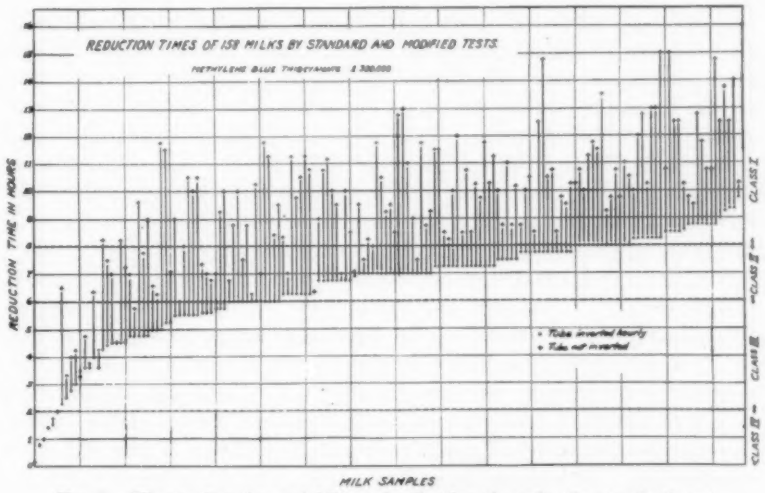

FIG. 1. Illustrating the variability of reduction times by the standard test.

author's data on 158 milks as presented in Fig. 1. Duplicate tubes were incubated together in the absence of light, one of each pair being inverted hourly,[6] the other left undisturbed. Since the modified test furnishes the more accurate and dependable indication of bacterial activity, the modified reduction times have been arranged in ascending order of magnitude and the corresponding standard reduction times plotted against them to show the degree of variability encountered. It will be seen that in the standard test there are wide variations in reduction time for milks showing identical reduction times by the modified test. For example, standard reduction times for the 12 samples reducing in 6 hours by the modified test range all the way from $6\frac{1}{4}$ to $11\frac{3}{4}$ hours. Recent findings in this laboratory suggest that such wide

[6] Studies to be reported in a subsequent paper had shown that there was no significant difference in reduction time between half-hourly and hourly inversion. Thornton (29) reports similar findings with 4 samples studied.

differences are not due entirely to the variability shown by replicate tubes in the standard test. As the data in Table 1 indicate, high grade milks from certain herds consistently show greater differences between standard and modified reduction times than do milks of similar quality furnished by other herds. The available evidence indicates that this is associated with the depth of cream layer and the rate of creaming during incubation. It would appear, therefore, that attempts to convert standard reduction times to corresponding modified reduction times according to the equivalent values[7] suggested by Thornton (29) would penalize some shippers while unduly favoring others. It is believed that these inherent differences furnish an added reason for the adoption of the modified test.

In addition to the lengthening of reduction time as milk supplies improve, there are several other considerations which increase the urgency of the need for a more reliable test. The first of these is the proposal to raise the limit for Class I milks from 5½ to 8 hours in the forthcoming 7th edition of Standard Methods of Milk Analysis. Since all investigators agree that the standard test becomes less reliable as the reduction time increases, this extension of the time limits will doubtless result in more serious errors in grading than under the old standards.[8] The reliability of the modified test, on the other hand, is not affected by the lengthening of reduction time. The second is the proposal to increase the dye concentration from 1 : 700,000 as now employed to 1 : 300,000 (26). Thornton and Sandin (26) report that with the higher dye concentration, reduction times are lengthened on the average by 30 minutes. Frayer (8) finds an increase in reduction time of the order of 100 per cent with the stronger dye concentration.[9] In the present studies it has been found that the increase is not a fixed increment for all classes of milk as reported by Thornton and Sandin but amounts to about 20 per cent of the reduction time. Data presented in Fig. 2 from comparative tests with single tubes inverted hourly illustrate this. While there are some exceptions, the general trends of the two curves show good agreement, the spread between the two sets of values widening as the reduction time lengthens. Since the bacteria were maintained in fairly uniform dispersion by hourly inversion, the occasional departures from the average are unlikely to be due to creaming.

[7] The equivalent values suggested are:

Standard reduction time	*Modified reduction time*
8: 00	6: 00
5: 30	4: 00
2: 00	2: 00

[8] In expressing the view in 1930 (16) that the standard reduction test was reasonably accurate up to 10 hours, the author had in mind its accuracy compared with that of the plate count in the examination of better class milks. Thornton (29) apparently holds the same opinion. It cannot, however, be regarded as equivalent in accuracy to the modified test beyond the first few hours.

[9] As subsequently mentioned, the technic adopted by Frayer may be responsible for the unusually great increase reported.

TABLE 1

Standard and modified reduction times of consecutive samplings from certain herds

Shipper's number	S	M	S	M	S	M	S	M	S	M	S	M	S	M	Ratio $\frac{S}{M}$
1 N	12: 15	6: 45	15: 00	8: 30	9: 15	6: 00	8: 45	6: 15	15: 00	7: 30					1.72
P	6: 30	4: 00	11: 45	6: 00					6: 45	5: 30					1.61
2 N	10: 00	5: 30	10: 15	5: 00	10: 00	5: 45	8: 15	4: 00	9: 15	5: 00	6: 45	5: 30			1.77
P	8: 30	4: 00	3: 15	2: 40	6: 45	3: 30			4: 45	2: 45	8: 00	4: 40			1.78
3 N	15: 00	8: 15	13: 00	6: 45	12: 45	7: 00	10: 15	6: 45	11: 30	8: 30					1.68
P	10: 10	6: 30	8: 15	5: 15	12: 00	5: 15			12: 00	7: 00					1.57
4 N	13: 00	8: 15	11: 45	5: 30	11: 45	7: 00	10: 45	5: 45	11: 15	6: 15	10: 00	6: 00	14: 00	4: 40	1.90
P	3: 15	2: 40	1: 40	1: 30	2: 50	2: 00	1: 20	1: 20	8: 15	3: 45			2: 30	2: 00	1.49
5 N	11: 15	6: 15	10: 15	5: 00	10: 45	6: 00	9: 15	5: 15	11: 00	7: 00	11: 00	6: 45	7: 45	4: 15	1.76
P	3: 45	2: 00	1: 30	1: 20	2: 50	2: 10	1: 10	1: 10	7: 00	4: 00			2: 00	1: 30	1.50
6 N	8: 00	7: 00	5: 15	4: 15	8: 30	7: 00	5: 45	4: 45	10: 45	8: 45	12: 00	10: 15	6: 30	5: 15	1.20
P	4: 45	3: 45	2: 40	2: 40	7: 00	4: 45	2: 50	2: 10	7: 15	5: 00			5: 30	3: 00	1.40
7 N	6: 15	6: 00	7: 15	5: 30	8: 30	8: 00	8: 00	7: 00	9: 15	8: 45	7: 00	6: 15	3: 15	3: 15	1.11
P	1: 20	1: 20	1: 00	0: 50	5: 30	5: 00	4: 00	4: 00	4: 45	4: 45			1: 00	1: 00	1.05
8 N	6: 45	6: 00	7: 00	6: 45	8: 15	7: 15	9: 30	7: 30	9: 45	8: 00	8: 30	7: 15	7: 15	5: 00	1.19
P	3: 30	3: 30			4: 10	4: 30	2: 40	2: 00	4: 00	3: 45	7: 30	5: 15	4: 45	3: 00	1.20
9 N	8: 15	7: 00	7: 30	6: 15	8: 30	7: 45	7: 15	6: 30	13: 45	9: 15	6: 00	5: 15	2: 30	2: 15	1.21
P	3: 45	3: 40	2: 00	2: 00	6: 15	5: 15			10: 15	7: 15			1: 00	0: 45	1.23

N.B. S = Standard Reduction time. M = Modified Reduction Time. N = No Preliminary Incubation. P = Preliminary Incubation at 12.8° C. for 18 hours.

It is more probable that differences in dye sensitiveness of the milk flora may be the cause.

At this point, mention should be made of some anomalous results encountered in 1934 when collaborating with Dr. H. J. Conn, Chairman of the Commission on Standardization of Biological Stains, in the testing out of the new methylene blue thiocyanate. With certain samples the modified test led to significant lengthening of the reduction time with the higher dye concentrations. This was not noted where the standard test was employed.[10]

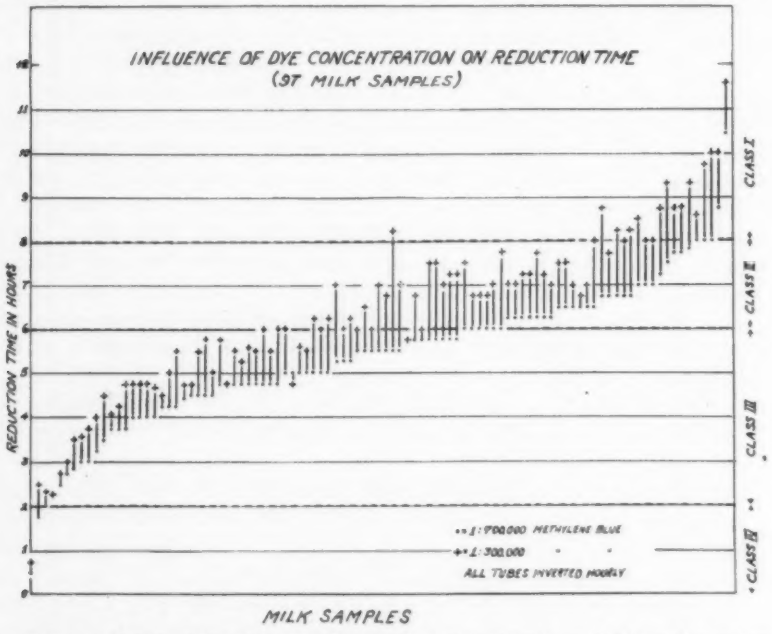

FIG. 2. Corresponding reduction times of 97 milk samples with old and new dye concentrations.

Similar cases have been encountered only twice during the present studies covering several hundred samples. Such cases would appear therefore to be too infrequent to deserve special consideration.

In passing, attention should be called to the fact that there is a much more satisfactory agreement between the modified reduction times with the two different dye concentrations (Fig. 2) than is the case with the standard and modified reduction times at either dye concentration (Fig. 1).[11] It will

[10] Thornton (30) has encountered two samples giving similar results.

[11] Results almost identical with those shown in Fig. 1, were obtained where a dye concentration of 1: 700,000 was employed.

also be noted that with the exception of Class IV milks the increase in reduction time resulting from the stronger dye concentration is considerably less than the average increase where creaming is permitted to take place. The mixing modification therefore more than compensates for the delayed reduction with the stronger dye concentration.

A further reason advanced by Thornton (29) for rejecting the modified test is that "it complicates the operation of the test to the extent that strictly uniform technic would be improbable in the hands of some who are at present satisfactorily performing the test." This was originally interpreted as referring to the *technic* of inverting the tubes but in commenting on the original draft of this paper Thornton (30) indicated that he had in mind (a) the difficulty of getting operators of the test, who often have various other duties to perform, to invert the tubes every hour, and (b) the test will have doubtful application in cheese factories, etc., if it is made the least bit more complex. Concerning the first point, it has been found in the present studies that any program of inversion, no matter how irregular the intervals, will increase the reliability of the test.[12] The slight inconvenience involved in periodical inversion of the tubes appears to be more than compensated for by the shortening of reduction time, simpler end-point determination, and greater reliability with the better grades of milk. As to the second point, the mixing modification is least valuable with the poorest grades of milk. For the ordinary run of cheese milks it will probably make little difference if inversion is omitted. On the other hand, where the test is used as the basis for premium payments for the best milks,[13] the more accurate technic should be employed. In the interests of uniformity and accuracy, therefore, it would seem desirable that the modified test replace the present technic for all classes of milks.

TECHNIC OF DETERMINING THE END-POINT IN THE MODIFIED TEST

Most investigators have recognized that reincorporation of oxygen immediately prior to reduction may unduly prolong the reduction time of certain milks and have modified their technic accordingly. Wilson (32, p. 216) specifies that "Any tube at the time of examination showing obvious signs of reduction should not be inverted, but should be left until the end-point as defined is reached." Thornton (30) employs a similar procedure, as does the present author. Frayer (8) does not state his procedure. That some judgment must be exercised by the operator of the test in deciding when to stop inverting the tubes is admitted. It is believed, however, that the difficulties involved are no greater, and probably far less, than those involved

[12] Thornton's own data (29, Table 6) show marked improvement resulting from inversion of tubes at the 4th, 6th and 8th hour of incubation.

[13] A program for improving cheese milk supplies along these lines is being considered at present in Wisconsin.

in attempting to estimate the end-points of the majority of better-class milks under the standard technic, where patchy and uneven decolorization of the dye is often encountered.

The method of determining the exact end-point with the reduction test is deserving of further study. Most investigators have worked out a technic for reading the end-point which satisfies them. That employed by the author is similar to that described by Wilson (32, p. 231), who remarks that "the point of 'complete reduction' of methylene blue is impossible to measure either visually or electrometrically." Frayer (8) on the other hand states that he has taken the point of complete reduction by transmitted light as the end-point but gives no details of his method. It seems not unlikely that this may be responsible for some of the differences between his results and those of other workers in comparing different dye concentrations, particularly if tubes were inverted without regard to incipient reduction.

SUMMARY

1. The methylene blue reduction test is based upon the rate of oxygen consumption in the milk by the bacteria present during incubation. This rate cannot be measured with reasonable accuracy where varying proportions of the bacteria are removed from the main body of the milk during the creaming process.

2. The accuracy of the test is greatly increased by periodical inversion of the tubes during incubation. In addition, reduction time is generally shortened and decolorization is more uniform, especially with the better grades of milk.

3. It is believed that these advantages more than compensate for the slight inconvenience entailed by this modification.

4. Milks showing similar reduction times by the modified technic sometimes show wide differences in standard reduction times. These appear to be associated with differences in the degree and rate of creaming.

5. Reduction time is prolonged by approximately 20 per cent with the proposed stronger dye concentration. This is more than compensated for in all but the poorer grades of milk by the shortening of reduction time when inversion is practised.

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THE INFLUENCE OF ACIDITY VARIATIONS DURING MANUFACTURE ON THE QUALITY AND RATE OF RIPENING OF BLUE OR AMERICAN ROQUEFORT CHEESE

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The limits of desirable acidity development at the various stages in the manufacture of cheddar cheese have been established by the results of numerous investigations. Comparatively little of such information is available to the manufacturer of Blue or American Roquefort cheese. No mention is made of acidity control in the reports of the methods used in France in the manufacture of Roquefort cheese (1, 2, 3). Funder (4), working in Norway on a modification for making Roquefort type cheese from cow's milk, recommended the addition of 2 to 2.5 per cent culture in order to secure mold vegetation. He considered a high acidity necessary for abundant growth of the penicillium.

Thom and Matheson (5) have given the subject considerable attention. According to their experiments cow's milk should be brought to an acidity of about 0.23 per cent by the addition of starter or by the use of a smaller amount of starter with a subsequent ripening period, before the addition of the rennet. The cheese from milk set at a lower acidity did not drain properly, were soft and developed off-flavors. Higher setting acidities were found to be detrimental to the texture of the curd; as tough, waxy or gummy textures frequently developed.

Matheson (6) later again recommended that the milk be set at an acidity of 0.21 to 0.23 per cent. Goss, Neilson and Mortensen (7), however, state that the rennet should be added when the milk reaches an acidity of 0.19 to 0.20 per cent. The actual amount of acidity due to added starter and to ripening, which doubtless is of more importance than the acidity of the milk itself, is not mentioned.

EXPERIMENTAL

In the manufacture of Blue cheese at the Dairy Division of the University of Minnesota, considerable variation in the rate of ripening of this cheese has been noted. Along with other factors which might influence the rate of ripening and the uniformity of the cheese, acidity variations during manufacture have been investigated. Variables studied included the amount of starter used, the extent to which the milk was ripened and the acidity of

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[1] The data in this paper are from a thesis presented by J. Spencer George in partial fulfillment of the requirements for the degree of M.S., University of Minnesota. Journal Series Paper 1548, Minnesota Agricultural Experiment Station.

the whey at dipping. The starter used was a culture of *Streptococcus lactis* with associated types.

In each of 5 replicated series, 4800 pounds of raw milk were used. The milk was divided into six lots. Three lots in each series were set at approximately the acidity to which the addition of 4 per cent starter would bring the milk. The other 3 lots were permitted to ripen, following the addition of starter, to an acidity about 0.03 to 0.04 per cent higher. The initial acidity of the milk in the different series varied from 0.15 to 0.20 per cent, causing an equivalent variation in the acidity at which the milk in the comparable lots in the different series was set. In most cases the milk in the low acidity group was set at 0.19 to 0.20 per cent and the high setting group at 0.23 to 0.24 per cent acidity. In each group of 3 vats, one vat was inoculated with 2 per cent starter, one with 3 per cent and the other with 4 per cent starter. Each vat was divided with respect to dipping acidity, half the vat being dipped soon after cutting, while the remainder was left in the whey until the acidity had increased 0.05 to 0.06 per cent. This required about one hour in most cases.

Every effort was made to standardize the composition of the cheese, particularly the salt content. The cheese were salted by rubbing salt over their surfaces. In three applications a total of 7.5 pounds of salt per 100 pounds of green cheese was used. This procedure has been found at this station to produce uniformly cheese containing about 5.5 per cent of salt at 3 months of age.

In addition to the customary manufacturing data, pH determinations, were made at the various stages during manufacture and ripening. To follow the cheese ripening, analyses were made for volatile fatty acids and amino nitrogen on the 2-day-old cheese and on the cheese at 3, 6 and 9 months. The cheese were examined for flavor, body, texture, and character of mold growth at 3 month intervals. The cheese also were analyzed for moisture, salt and fat. The pH determinations were made using a quinhydrone electrode with a Leeds and Northrop type K potentiometer. The method described by Currie (8) for the estimation of volatile acids was followed. Results are expressed as millimeters of N/10 acid per 100 grams of cheese. The amino nitrogen values were determined using the micro-Van Slyke gasometric method. Results are expressed as milligrams of nitrogen per gram of dried fat free cheese. One half cheese was ground and used for analysis at each period. Since the cheese are handled separately, individuality of the cheese assumes a considerable role in the variability of the data.

RESULTS

Only one important difference was observed in the cheese during the manufacturing period. The customary procedure is to remove the cheese

from the hoop after about 20 hours. In the first series the cheese made from the milk to which 2 per cent starter was added and set at low acidity were so soft that they flattened somewhat on removal from the hoop. This flattening has been observed in other cheese set at low acidity, particularly in instances in which an inactive starter was used. These results indicate that 2 per cent starter is about the minimum which should be used unless the milk is to be ripened considerably.

The amount of starter used does not appear to have significantly influenced any character of the cheese. This is in keeping with the results secured by Thom and Matheson (5) who found that it was immaterial whether the desired acidity was secured by the addition of starter alone or in part by ripening the milk subsequent to the addition of a smaller amount of starter.

TABLE 1

Influence of variation in setting and dipping acidity on the pH of the cheese at various intervals during ripening

Acidity during mfg. of cheese	Average pH of cheese at different stages of ripening			
	salting	3 months	6 months	9 months
Low setting Low dipping	4.76	6.24	5.98	5.82
Low setting High dipping	4.72	6.00	5.89	5.71
High setting Low dipping	4.79	6.04	5.92	5.63
High setting High dipping	4.74	5.98	5.86	5.60

Hydrogen Ion Concentration of Cheese

The acidity of the cheese as indicated by the hydrogen ion concentration appears to have been influenced at certain stages in the ripening of the cheese both by the acidity of the milk at setting and by the acidity of the whey at dipping. These results are summarized in Table 1. Examination of the data by analysis of variance shows that at salting there were no significant differences in the pH values of the cheese in the different acidity groups. At 3 and 6 months the average pH value for the cheese set and dipped at low acidity is somewhat greater than for the other cheese. Those cheese set and dipped at high acidity have the lowest average pH value. There differences are not statistically significant as the ratio of the greater to the lesser mean square is 3.5 at 3 months and 2.2 at 6 months. According to Snedecor (9) unless the value of F (ratio of the greater to the lesser mean square) for this number of observations is equal to or greater than 4.20 there are more than five chances in a hundred that there is no real difference in the data.

By the time the cheese were 9 months of age, however, variations in the setting acidity and in the dipping acidity appear to have caused significant

differences in the acidity of the cheese. The cheese set and dipped at low acidity have the highest and those set and dipped at high acidity the lowest average pH values. The ratio of the greater to the lesser mean square is 22.2 which is far outside of the range of Snedecor's highly significant value, which for this number of observations is 7.64. This indicates that there is much less than one chance in a hundred that there is no real difference in the data.

With high setting acidity, variation in the dipping acidity did not significantly influence the pH of the cheese at 9 months. The difference in the average pH values for the cheese dipped at high and low acidities in the low setting acidity group, however, is highly significant. Setting acidity appears to have affected the pH values of the cheese at this period irrespective of whether the dipping acidity was high or low. The difference, however, is slightly greater for the low dipping acidity group.

The Rate of Ripening

A summary of the data on fat hydrolysis and protein degradation is shown in Figure 1. The values shown are the means for the 60 cheese at each period of analysis. The increase in the volatile fatty acids and amino nitrogen with time is apparently essentially a straight-line relationship.

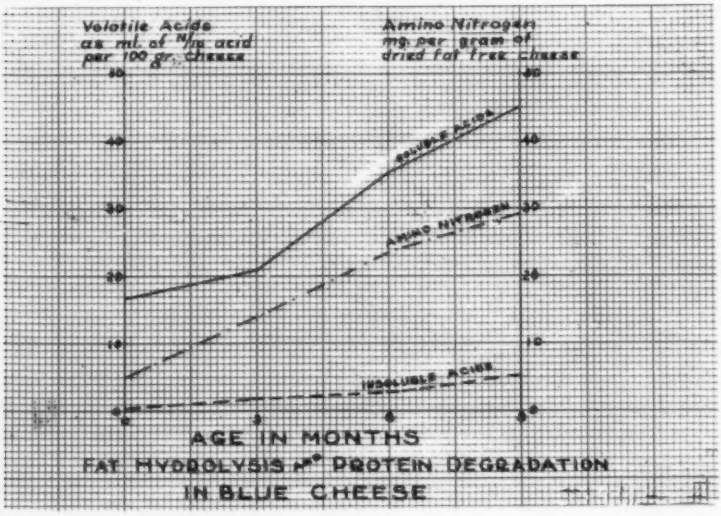

FIG. 1.

The mean values for soluble and insoluble volatile acids and amino nitrogen grouped according to setting and dipping acidity are shown in Table 2.

A low dipping acidity especially when combined with low setting acidity appears to favor fat hydrolysis and protein degradation. The differences between the values for those cheese for which the curd was dipped at low acidity and those dipped at high acidity in the low setting acidity group are significant or highly significant as shown by the values for F, except for the amino nitrogen values at 6 and 9 months and the soluble volatile acids at 3 months. The same trend is apparent at these periods, although the values of F are not sufficiently great to indicate a significant difference.

The mean values for volatile acids and amino nitrogen for the cheese dipped at low acidity are also higher than those for the cheese dipped at high acidity in the high setting acidity group. The F values indicate, however, that there are somewhat more than five chances in a hundred that these differences are due to chance.

For those cheese for which the dipping acidity was high, whether the milk was set at low or at high acidity, there appears to have been no effect on the rate of ripening. The mean values for volatile acids and amino nitrogen are similar at every period. A faster rate of ripening appears to have occurred in those cheese set at low acidity than in those set at high acidity when the dipping acidity was low. These differences are not large, nor very uniform, but do indicate a trend.

Relation of pH to Rate of Ripening

Considering the data as a whole there does not appear to be any consistent difference in the amount of soluble and insoluble volatile acids and amino nitrogen at the various periods attributable to differences in the hydrogen ion concentration of the cheese. As noted previously only at 9 months was there any significant difference in the pH of the cheese in the various acidity groups. At this period the mean pH of the cheese set and dipped at low acidity was very significantly higher than the mean pH of those set and dipped at high acidity. At this period also, the volatile soluble acids and amino nitrogen values for the group set and dipped at low acidity were very significantly higher. The mean value for volatile insoluble acids was higher but not significantly so. The correlation coefficient between the pH and amino nitrogen values for the cheese in these groups at this period is $+0.82 \pm .06$, between pH and volatile soluble acids $+0.69 \pm .096$, and between pH and volatile insoluble acids $+0.29 \pm .17$. Thus there seems to be a definite association between pH and amino nitrogen and pH and volatile soluble acids in the cheese at 9 months. Whether the volatile acidity and amino nitrogen are low because ripening was retarded by low pH, or whether pH is high because of changes in pH due to decomposition products, it is impossible to say.

TABLE 2

The influence of high and low dipping acidity and high and low setting acidity on fat hydrolysis and protein degradation of Blue cheese

Acidity at Dipping	Volatile Acids						Amino Nitrogen		
	Soluble			Insoluble					
	3 mo.	6 mo.	9 mo.	3 mo.	6 mo.	9 mo.	3 mo.	6 mo.	9 mo.
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*mg.*	*mg.*	*mg.*
Low Setting Acidity									
Low	21.87	41.43	57.29	2.25	3.39	6.03	15.65	24.60	30.85
High	17.60	32.21	39.31	1.60	2.63	4.61	13.86	23.53	29.24
Value of F.*	3.30	10.51	11.30	4.17	7.24	8.52	6.34	1.68	2.27
High Setting Acidity									
Low	23.37	36.48	44.52	2.13	3.17	5.20	14.05	23.64	28.46
High	21.27	32.33	39.24	2.01	2.68	4.96	12.20	22.50	27.64
Value of F.*	1.20	2.59	1.98	0.22	2.07	0.12	4.09	1.28	0.67

* For this number of observations (30) a value of F. as great as 4.20 indicates a significant difference, a value as great as 7.64 a highly significant difference [Snedecor (9)].

Quality of Cheese

Variation in acidity during manufacture did not significantly influence the grade of the cheese but did influence to some extent the occurrence of certain flavors in the cheese. In general high acidities during manufacture resulted in more acidy flavored but less musty flavored cheese. Firm bodied and crumbly cheese occurred more frequently where high acidities were used. The cheese set and dipped at low acidities were ready for market somewhat sooner than those set or dipped at high acidity. This would be expected considering the effect of low dipping and low setting acidity on the rate of fat hydrolysis and protein degradation. These cheese, however, deteriorated in flavor sooner. The cheese in the manufacture of which high acidities were used ripened later, but many of these were sold as excellent cheese when over one year old.

SUMMARY AND CONCLUSIONS

Blue cheese were manufactured using 2, 3 and 4 per cent starter, with the milk set at low acidity (0.19 to 0.20 per cent), and after ripening to 0.23 to 0.24 per cent, with the curd dipped after cutting before additional acidity development, and with dipping delayed until 0.05 to 0.06 per cent acidity developed in the whey.

Variations to this extent in acidity during manufacture do not appear to be highly important in the manufacture of Blue cheese. Excellent cheese were produced with all combinations used. Cheese set and dipped at low acidity as indicated by fat hydrolysis, protein degradation and character of the cheese, ripened somewhat sooner than those set and dipped at high acidity.

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American Dairy Science Association Announcements

AMERICAN DAIRY SCIENCE ASSOCIATION ANNUAL MEETING JUNE 14–17, COLUMBUS AND WOOSTER, OHIO

HOUSING PLANS

The University girls' dormitories located on the campus will be available beginning June 14 for approximately 350 people at a cost of $1.00 per person per night. The rate for children will be the same as for adults. Many of the rooms have connecting baths and all facilities are good. The dormitories are within one block of the place of meetings. Rooms will also be available at special rates in Columbus hotels. Members will receive full details regarding housing in a letter to be sent out in early May. All reservations and further information on housing can be obtained from the chairman of the Housing and Registration Committee, J. H. Erb, Dept. of Dairy Technology, Ohio State University, Columbus, Ohio.

GOLF AT THE CONVENTION

It is hoped that those in attendance at the Annual Meeting will take advantage of the excellent golf facilities at the University. The University is opening a new course this spring which will be available at a nominal cost.

Make your plans now to bring your family and attend the annual meeting.

JOURNAL CIRCULATION

The officers of your Association are attempting to increase the circulation of the JOURNAL OF DAIRY SCIENCE to 2000.

In 1936 the officers made an agreement with the International Association of Milk Dealers and the International Association of Ice Cream Manufacturers to publish abstracts of literature on milk and milk products. The two Associations agreed to promote the circulation of the JOURNAL. The circulation was increased a little more than 400. It reached about 1785 in 1936.

In 1937 there were 958 members, 745 subscribers and 111 associate subscribers making a total circulation of 1813.

Each year we have about 300 who drop their membership or subscriptions. In order to get our circulation to 2000, it will be necessary to increase the membership and subscribers by about 500.

By the fifteenth of March, 790 of the 1937 members had paid their dues, 539 of the subscribers had renewed, and 70 of the associate subscribers had renewed for 1938. Many of the delinquent members and subscribers will, we trust, pay up in 1938. We now have 107 new members, 82 new subscribers, and 49 new associate subscribers, making a total circulation of 1637.

We will appreciate your requesting subscription order blanks or application blanks to send to prospective members and subscribers.

We should have more commercial breeders and those interested in dairy production as our readers. It will be necessary to increase our circulation to carry the additional cost of production abstracts.

We are sorry to announce the passing of Prof. Rush B. Locke on January 27, 1938. Professor Locke had charge of the work in dairy manufacturing at the Colorado State College, Fort Collins, Colo. He had been ill for the past six months.

Prof. Rush B. Locke was born April 22, 1898. He attended South Dakota State College from which he received the bachelor of science degree in June, 1924. In 1925 and 1926 he occupied a teaching fellowship at Iowa State College from which institution he received the degree of master of science in 1926. From 1926 until 1928 he was an instructor in the Department of Dairy Manufactures at Iowa State College. In 1928 he came to the Colorado State College as associate professor of dairy manufactures.

In August, 1937, he went to Fitzsimmons General Hospital (U. S. Army), Denver, Colorado, for observation. He remained there almost continuously, with the exception of a few happy week-ends at home, until his death January 26, 1938, when he died of nephritis and heart disease. Surviving him are Mrs. Locke and their three sons, Rush, Jr., 11 years of age, Richard, 9, and David, 7. Locke was much admired and respected by his friends on our campus and had played an especially important role by establishing and managing a dairy short course of several days duration each year.

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

123. Standardization of Tablets for Determining Methylene Blue Reduction in Milk. H. J. Conn, N. Y. Agr. Exp. Sta., Geneva, N. Y. Am. J. Pub. Health *27*, 8, p. 793, 1937.

The variation in the dye content of Danish and American tablets is discussed. Methylene blue thiocyanate is preferable to methylene blue chloride. Methylene blue thiocyanate tablets are now being placed upon the market with the approval of the Committee on Standard Methods of Milk Analysis of the American Public Health Association. It is believed that American manufacturers will be able to put out a very uniform product.

M.W.Y.

124. Influence of Contaminating Bacteria on the Results of the Microscopic Test for Streptococci Mastitis. C. S. Bryan and E. A. Nelson, Michigan Agr. Exp. Sta., East Lansing, Michigan. Am. J. Pub. Health *27*, 9, p. 914, 1937.

Placing 0.1 cc. of a sterile 1–500 aqueous dilution of brilliant green into each tube prior to collecting a 10 cc. sample from the udder is recommended as a method of inhibiting contaminants. M.W.Y.

125. Influence of Dead Bacteria on Microscopic Counts of Pasteurized Milk. Archibald R. Ward and Charles E. Myers, Dairy Testing Lab., Detroit, Michigan. Am. J. Pub. Health *27*, 9, p. 899, 1937.

The widely held belief that bacteria killed by pasteurization remain visible and are counted together with those remaining alive is not accepted by the author. Microscopic counts were made from milk held for varying lengths of time at 142° to 144° F. The author concludes that insufficient numbers of dead bacteria remain visible after pasteurization to impair the usefulness of direct microscopic counts made on pasteurized milk.

M.W.Y.

126. Modified Methylene Blue Reduction Technic. H. R. Thornton, Univ. of Alberta, Alberta, Canada. Am. J. Pub. Health *27*, 8, p. 791, 1937.

Advantages and disadvantages of shaking the tubes of milk during incubation, the standard technic in Great Britain, are discussed. A comparison of the standard and modified tests on 332 market milks showed that neither test greatly excelled the other in accuracy, despite the greater variability

of the standard test. The author finds no justifications for adopting the modified test as standard on this continent. M.W.Y.

127. The Need of Uniformity of Conditions for Counting Plates with a Suggestion for a Standard Colony Counter. JACQUES ARCHAMBAULT, J. CUROT, AND MAC H. MCCRADY, Div. of Lab., Quebec Ministry of Health, Montreal, Quebec. Amer. J. Pub. Health *27*, 8, p. 809, 1937.

A counter for enumerating bacterial colonies on agar plates prepared from milk, water and other materials is described. The "Quebec Colony Counter" combines the essential features which makes for constant magnification and illumination, for the convenience and comfort of the operator, and for accuracy of counts. M.W.Y.

128. The Method of Frost Modified by Van Oijen. H. BARKWORTH, Special Dairy Bact., College of Agr. of the Southeast, Wye, Kent. Le Lait *17*, 168, p. 810, Sept.–Oct., 1937.

The modified method of Van Oijen was compared with the standard plate count procedure for determining the bacterial count of milk. The Van Oijen slides were incubated at 28° C. (82.4° F.) for 24 hours. The Van Oijen procedure was considered more precise than the plate count procedure for determining the bacterial count of milk. Details of the Van Oijen procedure are given. A.H.J.

129. The Colon Bacilli Sensitive to Bacteriophage. IRENE LIPSKA, Municipal Inst. of Hygiene, Warsaw, Poland. Le Lait *17*, 169, p. 913, Nov., 1937.

Of the sensitive colon bacilli studied, 68.6 per cent of the strains had chemical properties characterizing them as fecal strains. Following the classification, *Bact. coli commune* had 31.4 per cent of sensitive strains. The greatest frequency of sensitive strains (100 per cent) was among the birds and the marine invertebrates. The vigor of development at 22° C. (71.6° F.) and at 37° C. (98.6° F.) as well as the vitality of the strain as shown by the rate at which carbohydrates are fermented are inverse to the sensitivity of attack by bacteriophages. The sensitive strains of colon bacilli studied are characterized by a great constancy in their properties comprising more or less high resistance to bacteriophage. A.H.J.

BUTTER

130. Differences in the Lactic Acid Percentages in Butters. E. O. WHITTIER AND C. S. TRIMBLE, Bureau of Dairy Industry, Washington, D. C. Am. Creamery and Poultry Prod. Rev. *81*, 15, p. 518, Feb. 12, 1937.

Butter made from cream containing less than 0.25 acid and no starter will contain less than 0.025 apparent lactic acid; cream with 0.4 or greater per cent acid and no neutralizer will produce butter with 0.1 or greater per cent acid; sour cream neutralized to 0.2 per cent acid will contain 0.15 or more acid; cream treated with water or sweet skimmilk or washed with alkaline water will show slightly decreased acid content; and storage of butter at somewhat higher temperatures than those usually used will show no increase in apparent acid content. P.S.L.

131. **Progress in the Cream Improvement Movement.** J. O. CLARKE, Food and Drug Administration. Am. Creamery and Poultry Prod. Rev. *83*, 15, p. 504, Feb. 10, 1937.

In 1935, 1936, and 1937 the per cents of cans of cream condemned were 4.00, 3.06, and 2.88 respectively. The program of improvement has just begun; better tests for measuring fitness of cream are needed. The sediment test does not measure decomposition; taste and odor standards are indefinite. Acidity, hydrogen-ion concentration and formol tests, and mold counts are being used to a greater degree. The first is used indirectly as an index of conditions of sanitation. The second may be impractical for creamery use since neutralizer nullifies the results. This is not true for the formol test. It is of value in judging relative degree of decomposition. A special test has been perfected for relative mold contamination and has given good results. Farm sanitation and frequent delivery of cream are objectives in any good cream improvement program. P.S.L.

132. **Laboratory Manual of Methods of Analysis for the Butter Industry,** by the Research Committee (M. E. PARKER, *chairman*) of the American Butter Institute, 110 North Franklin St., Chicago, Ill.

This manual was designed to give the practical analytical procedures which may be used in a good creamery laboratory and which are used in the laboratory of the American Butter Institute. The official control methods are not included, for they are generally not adaptable to plant work.

The various chapters presented are on butter sampling; determination of yeasts and molds, moisture, fat, salt, and curd; pH; diacetyl and acetylmethylcarbinol; sediment testing of milk, cream, and butter; titratable acidity; the Babcock test; keeping quality of butter; and checking acidity and salt test solutions.

The manual has been printed only on one side of the paper and it opens flat on the dest. The style is such as to be of greatest use in the creamery laboratory. A.C.D.

133. **Delaying Oxidative Changes in Butter.** C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State College, State College, Pa. Nat. Butter and Cheese J. *28*, 18, p. 18, Sept. 25, 1937.

A water extract of oat flour (Avenex No. 7) added to sweet or sour cream before pasteurization improved the keeping quality of the butter without imparting off flavor or incorporating sediment. The weight of Avenex used equaled 1 per cent of the fat. This amount seemed to give ample protection against oxidative deterioration of butter during storage for two months at 40 to 45° F. W.V.P.

134. Butter Color and Vitamin A. K. G. WECKEL, Univ. of Wisconsin, Madison, Wisconsin. Nat. Butter and Cheese J. *28*, 17, p. 28, Sept. 10, 1937.

Natural yellow color of butter is due chiefly to carotene which in the animal body is transformed to vitamin A. A graph shows seasonal variations in carotene, vitamin A, and vitamin A activity in butter. Carotene accounts for 15 per cent of total vitamin A activity. There is little difference in total vitamin A activity of fat from four common breeds of cows when on the same ration, although carotene values vary widely. Feeds rich in carotene are listed and butter manufacturers are urged to encourage their use in order to improve nutritive value of butter. W.V.P.

135. Stabilization of Butter Against Oxidized Flavor. VIRGIL L. KOENIG, Okla. Agr. Exp. Sta., Stillwater, Oklahoma. Nat. Butter and Cheese J. *28*, 15, p. 26, Aug. 10, 1937.

Butter prints wrapped in parchment paper which had been treated with oat flour (Avenized Parchment) and butter mixed with 0.06 per cent of a hexane extract of oat flour (Avenol) and then wrapped in ordinary parchment were stored at 0° F. and at 50° F. for seventeen weeks. Avenized parchment and Avenol showed definite protective action against tallowiness and rancidity development. Avenol gave an off flavor to the butter during the first four weeks of storage. W.V.P.

136. Improving the Keeping Quality of Butter with Treated Parchment. C. D. DAHLE AND D. V. JOSEPHSON, Dairy Dept., Penn. State College, State College, Pa. Nat. Butter and Cheese J. *28*, p. 6, July 25, 1937.

Prints of butter wrapped in Avenex-treated parchment kept better at 45° F. and and slightly better at −15° F. than butter wrapped in ordinary parchment. W.V.P.

137. Keeping Down the Yeast and Mold Counts. A. E. GROTH, Federal-State Butter Grader, Mankato, Minn. Am. Creamery and Poultry Prod. Rev. *83*, 18, p. 702, March 10, 1937.

Mold spores may be kept down by keeping the creamery dry as with the use of overhead heating unit systems together with proper ventilation; by

frequent cleansing of the buttermilk and water storage tanks; keeping the covers of vats closed during pasteurization so as to pasteurize foam; drawing cream from gates of vats when heated and pouring into main body of cream; elimination of "dead pockets" in vats; checking seams and stuffing boxes frequently for contamination; thorough cleansing of pipe lines and pumps; especially thorough cleansing of the churn, for this is one of the chief sources of mold. Directions are given for care of the churn. P.S.L.

138. Carotene in Butter Coloring. R. B. Stoltz and T. S. Sutton, Ohio State University, Columbus, Ohio. Am. Creamery and Poultry Prod. Rev. *84*, 20, p. 614, Sept. 8, 1937.

The study concerns the use of semi-purified carotene in oil as a coloring material for winter butter, and as a method of increasing vitamin A content. Fifteen drops of Smaco carotene oil was added to a pound of butter and this was checked against a like sample containing no added carotene. Rats fed on the first sample showed a superior growth. Using a second group of rats, ¾ pound of butter colored with another brand of carotene produced as much growth as a group fed untreated winter butter. It was found impractical to raise the vitamin A content of winter butter, however, to that of June butter. In the latter case, using Primatone, it was necessary to use four ounces per 100 pounds butterfat to get a desired color. P.S.L.

Other Abstracts of interest are numbers 148, 182, 201, 207, 208, 209, 215, 216, 217, 218, and 219.

CHEESE

139. Modifications in the Composition of Cheese in the Course of Prolonged Storage. Jean Pien and G. Maurice, Lab. of the Farmers' Union, Paris, France. Le Lait *17*, 170, p. 1040, Dec., 1937.

The moisture content of Camembert cheese diminished regularly from 53 to 15 per cent during 13 months storage. During this period a considerable loss in dry matter also took place as a result of fermentation. This loss can attain 15 per cent of the original dry matter in the cheese and can accordingly cause a considerable increase in percentage of fatty matter in the moisture-free cheese, as the fatty matter is not lost by fermentation. During storage of the cheese for 13 months, the soluble nitrogen attained a value of 41.5 per cent of the total nitrogen and ammonia nitrogen was 60 per cent of the soluble nitrogen. During the storage period the total nitrogen of the cheese decreased from 6.36 per cent to 5.58 per cent, the soluble nitrogen increased from 0.55 per cent to 2.20 per cent and the ammonia content from 0.17 per cent to 1.25 per cent. A considerable percentage of ammonia nitrogen is lost from the cheese at the same time that other volatile matter results from the processes of fermentation. The losses that have been

described occurred when the cheeses were stored at a temperature of 8 to 10° C. (46.4° to 50° F.). A.H.J.

140. On the Change of the Lactose Content of the Curd after the Addition of Water in Making Edam Cheese. H. A. SIRKS, Report of the Experimental Dairy Farm, Hoorn, Holland, pp. 159–174, 1937 (English summary).

The cheesemaker often adds water to the whey during the cheesemaking process to lower the lactose content of the curd. In this study it was found that at least 15 minutes must be allowed after the addition of the water before the whey is drained off the curd, to make certain that a lactose equilibrium has been reached between that in the curd and the whey. A.C.D.

141. The Influence of Salt on the Composition and Quality of Brick Cheese. E. L. BYERS AND W. V. PRICE, Univ. of Wisconsin, Madison, Wisconsin. Nat. Butter and Cheese J. *28*, 14, p. 10, July 25, 1937.

A salt content of approximately 2 per cent by weight of the cheese seemed best. Less salt encouraged abnormal fermentation, high moisture, weak body and open texture. Excessive salting caused hard, harsh body, unnaturally white color, low moisture, loss of yield, delayed lactose fermentation and slow ripening. W.V.P.

142. Identification of Roquefort Cheese. IRA D. GARARD, ABRAHAM MINSKY, JAMES H. BAKER, AND VIOLA PASCALE, New Jersey College for Women, Rutgers Univ., New Brunswick, N. J., and Gar-Baker Lab., Inc., New York, N. Y. Ind. and Eng. Chem. *29*, p. 1167, Oct., 1937.

The fraudulent substitution of cow's milk Blue cheese for the more expensive ewe's milk Roquefort cheese makes desirable an effective means of differentiation. The color of the fat and its Polenske value provide a reliable method. Analyses of the fat of numerous authentic Roquefort cheeses and of a wide variety of cow's milk cheeses are reported. Polenske values for sheep milk fat varied between 3.6 and 5.95, while those for cow's milk fat never exceeded 2.9. The color of the extracted fat from cow's milk cheese was always deep yellow; that from ewe's milk fat was always pale green. The diet of the animals was found not to be responsible for the difference in range of Polenske values. The Polenske number does not change with the age of Roquefort cheese. J.H.N.

143. The Fermentation of the Egyptian Cheese "Mich." EL GHERIANY MOSTAFA, Bact. of the Dairy Service in the Lab. of Vet. Path., Guizeh, Egypt. Le Lait *17*, 168, p. 819, Sept.–Oct., 1937.

Bacillus saccharobutyricus amylobacter and *Streptococcus faecium* were regularly identified in "Mich." Often but not regularly "Mich" also contained *Bacillus mesentericus, Bacillus subtilis, Proteus ichthyosmus* and *Flavobacterium butyri.* On aging from 5 months to 14 months, the pH increased from 4.96 to 5.41, and the volatile acidity from 46 to 80 degrees Soxhlet. During the same period the butyric acid increased from 1.01 to 1.76 grams per 100 grams of the cheese. A.H.J.

Other Abstracts of interest are numbers 123, 126, 182, 207, 208, 209, 215, 216, 217, 218, and 219.

CHEMISTRY

144. The Physical and Chemical Properties of Casein Fat. SAMUEL G. STEVENSON AND ALFRED L. BACHARCH, Glaxo Lab., Ltd., Greenford, Middlesex, England. Biochem. J. *31*, 721, 1937.

It was reported in 1924 by other investigators that an anhydride of a hydroxystearic acid was obtained by repeated extraction of the residual fat occluded with casein separated from skimmilk.

The present authors investigated further the character of the fat extracted from lactic acid casein subsequently employed in preparing "fat-free caseinogen" used in Vitamin A-free basal diets. A comparison of the fat constants of the extracted fat with those for normal butterfat indicates that the casein fat is for the greater part indistinguishable from butterfat. A threefold concentration of unsaponifiable matter in the casein following purely mechanical separation of most of the milk fat is of interest and may be of physiological significance. An absence of phosphorus and consequently phospholipins in the extract was noted. K.G.W.

145. Metallic Content of Foods an Important Health Matter. A. H. STAND, Consulting Chemist, New York City. Cert. Milk *11*, 120, p. 7, April, 1936.

This article describes briefly spectrographic analysis as a new technique for a rapid qualitative and quantitative determination of metals in dairy products. By this method of analysis the author demonstrates the presence of tin in evaporated milk and the dissolving effect of cheese on tin foil. W.S.M.

146. The Value of the Volumetric Formol Procedure for Determining the Protein Content of Milk. B. VAN DER BURG AND L. HABERS, Dairy Lab. of the Agr. Univ., Wageningue, Holland. Le Lait *17*, 168, p. 805, Sept.–Oct., 1937.

The determination of the protein content of milk by the formol titration procedure was not found to have the required accuracy. A.H.J.

147. The Determination of Carotene in Forage. G. GENIN, Chemical Eng., Paris, France. Le Lait, *17*, 169, p. 727, Nov., 1937.

The author discusses the modifications introduced by Peterson, Hughes and Freeman into Guilbert's method for determining carotene. A.H.J.

148. The Determination of pH in the Service of Milk and Dairy Products Industry. ALFRED KARSTEN. Le Lait *17*, 169, p. 918, Nov., 1937.

Recently developed colorimetric and electrometric methods of determining the hydrogen ion concentration of dairy products are discussed. Photographs of the electrometric apparatus are shown. The significance of pH value in various branches of the dairy industry is discussed. Thus fresh milk outside the normal pH range 6.4 to 6.6 may indicate that it came from diseased cows. The use of pH control in the selection of milk for evaporated milk, milk powder as well as in the manufacture of butter and casein is discussed. A.H.J.

149. Measuring Milk Film. H. H. BECK, K. G. WECKEL, AND H. C. JACKSON, Univ. of Wis., Madison, Wisconsin. Am. Creamery and Poultry Prod. Rev. *83*, 13, p. 444, Jan. 27, 1937.

Thickness of the film of milk passing over irradiation apparatus and its speed of travel are factors in the determination of the degree to which it is subjected to ultra violet light, and should be of value to designers of apparatus for irradiation. Thickness of milk film was measured by light. A narrow beam of light was thrown at an angle of incidence of 45° on the metal surface and the reflected beam measured. The reflected beam was again measured while a film of milk was run over the surface. The distance between the two parallel beams of reflected light was determined and from this the thickness of the film calculated by geometrical formula.

P.S.L.

150. The Composition of Rabbit Milk Stimulated by the Lactogenic Hormone. A. J. BERGMAN AND C. W. TURNER, Univ. of Missouri, Columbia, Mo. J. Biol. Chem. *120*, 21, 1937.

The composition of rabbit milk obtained at various intervals after normal parturition was compared with the milk obtained from pseudo-pregnant rabbits in which lactation had been induced experimentally by the injection of lactogenic hormone for six days.

The amount of lactose and total solids in the experimental milk obtained from a rabbit having a 4+ rating (Gardner and Turner, in which the gland contains 0.76 to 1.0 per cent of lactose) were similar to that in the normal milk. The amount of fat was higher, and the ash content lower, in the experimental milk. K.G.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

151. Observations on Concentrated Frozen Milk. F. J. DOAN AND C. E. FEATHERMAN, Division of Dairy Mfgrs., Penn. State College, State College, Pa. Milk Dealer *27*, 2, p. 33, 62, Nov., 1937.

A report of studies on the concentration and freezing of milk as a means of preserving the product for a period of several weeks. The following factors were studied: (a) Degree of concentration, (b) homogenization, (c) length of holding period, (d) temperature of thawing.

The authors concluded that:

In concentrating and freezing milk for storage purposes a degree of concentration of approximately 3 to 1 appears to give most satisfactory results.

Homogenization prevents a rise of cream in the thawed, reconstituted milk. It decreases the tendency to churn, oiling-off and form cream plugs and also appears to aid in preventing tallowy flavor.

The length of time milk concentrated 3 to 1 can be held in a frozen condition and still produce a normal reconstituted product is somewhat indefinite and probably varies with the qualities of the original milk, the equipment used and the method of handling. From these results a storage period of 12 weeks seems quite possible.

The protein of concentrated frozen milk is apparently denatured by holding for long periods in the frozen state. This, however, appears to be a reversible action. In one sample encountered in these studies, protein which was apparently badly coagulated was re-dispersed in normal fashion by heating to temperatures between 150 and 160° F.

The thawing temperature does not exercise much influence on the properties of the reconstituted, concentrated frozen milk but a temperature of 120° F. appears to give less trouble in thawing and reconstituting than room temperatures of 50° F.

The number of trials made in this study and the number of samples included are too few to warrant very positive conclusions and it is hoped that the study may be continued in more detail, particularly with respect to the type of protein coagulation obtained when concentrated milk is held in the frozen state and with respect to the effect of concentration and freezing upon the curd tension. C.J.B.

152. Chemists Produce "Wool" from Milk. Home Economics Dept., Ohio Agr. Exp. Sta., Columbus, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. No. 22, 44, Jan. 6, 1938.

A brief description of the Italian process is given and a prediction made that "wool" from milk may develop comparably to rayon. W.E.K.

153. The Concentration and Properties of Vitamin H. LELA H. BOOKER, Dept. of Chemistry, Columbia University, New York. J. Biol. Chem. *119*, 223, 1937.

Methods for separating the relatively heat stable Vitamin H of the Vitamin B complex from low lactose whey powder, and from rice polishings is described. By the methods there is obtained 30 fold and 60 to 90 fold increase in concentration of the factor present in the whey powder and rice polishings, respectively. K.G.W.

154. Casein in the Fabrication of Plastic Materials. G. GENIN, Paris, France. Le Lait *17*, 168, p. 815, Sept.–Oct., 1937.

Casein precipitated by rennin is required for the making of casein plastics. A review is given of the methods for making casein plastics. Uses of casein plastics are discussed. A.H.J.

155. The Fabrication of Artificial Wool. G. GENIN, Chemical Engineer, Paris, France. Le Lait *17*, 169, p. 949, Nov., 1937.

A general discussion is given of the manufacture of casein and the fabrication of artificial wool fibers (Lanital) therefrom. Comparative carbon, hydrogen, oxygen, nitrogen and sulphur contents of true wool and Lanital are given. Except for the sulphur content which is higher in wool and the carbon content which is higher in Lanital, the composition of the two substances is much the same. In cold caustic soda Lanital dissolves more rapidly than wool. On burning wool and Lanital fibers, the residues are much the same. On boiling a sample of Lanital for 3 hours in an alkaline soap solution, it loses about 1 per cent of its weight, while on treating wool in the same manner, the loss in weight is about 10 times as great. Lanital is readily dyed. It is felted more readily than wool. A.H.J.

Other abstracts of interest are numbers 123, 124, 125, 126, 127, 132, 133, 134, 135, 136, 144, 145, 148, 149, 150, 157, 158, 159, 164, 165, 175, 176, 182, 188, 197, 199, 201, 202, 206, 207, 208, 209, 211, 212, 215, 216, 217, 218, and 219.

DISEASES

156. Bovine Tuberculosis. Editorial. Am. J. Pub. Health, *27*, 9, p. 919, 1937.

This editorial describes an outbreak of tuberculosis in a Swedish rural community in which 50 persons, mostly children, suddenly showed signs of tuberculosis. Practically all had been supplied with raw milk from one dairy. One cow which had been passed as free from tuberculosis upon clinical examination by veterinarians had an acute tuberculosis of the udder which resembled the usual type due to streptococcus. However, the milk

teemed with tubercle bacilli. Clinical examination of milk cows, even by the most skillful veterinary surgeon, is not sufficient. "Not only should milch cows have the tuberculin test, but all market milk should be pasteurized, and this applies even to what we know in this country as Certified milk" states the editorial. M.W.Y.

157. A Milk-Borne Epidemic and Its Lesson. Editorial. Am. J. Pub. Health, *27*, 10, p. 1042, 1937.

Only pasteurization of all market milk can avert such tragedies as the outbreak of typhoid fever in England which is described. Approximately 718 persons contracted the disease, 31 cases being fatal. Pasteurization was immediately successful in bringing the outbreak to an end. M.W.Y.

158. Vaccination Against Bang's Disease in an Infected Dairy Herd with United States Bureau of Animal Industry Brucella Abortus Strain 19. C. M. Haring, University of California, Berkeley, Calif. J. Am. Vet. Med. Assn., N.S. *45*-1, p. 52, Jan., 1938.

In a test of the resistance of dairy cattle to Brucella infection, 93 heifers were vaccinated with vaccine prepared from strain 19. During a four year period in which these heifers were in a dairy herd affected with brucellosis, this treatment apparently has been beneficial in retarding the spread of the disease. Strain 19 was not spread to healthy non-vaccinated cattle through association with vaccinated animals. Vaccine from strain 19 used on four cows induced a pronounced but temporary drop in milk production. Vaccination during advanced pregnancy of a cow kept in isolation brought about a typical brucellosis. J.W.W.

159. Consider Vaccines for Bang's Disease. B. H. Edgington, Ohio Agr. Exp. Sta., Animal Disease Control Laboratory, Reynoldsburg, O. Ohio Agr. Exp. Sta., Weekly Press Bull. *22*, 45, Jan. 13, 1938.

Indiscriminate use of vaccines is not justified in any known scheme of Bang's disease control. Calfhood vaccination seems to have merit, although its use is still in the experimental stage. The use of vaccines appears most warranted in infected herds where removal of reactor cattle or adequate segregation of reactor and non-reactor animals is impractical. W.E.K.

FEEDS AND FEEDING

160. Preparation and Nutritive Value of A. I. V. Alfalfa Silage for Dairy Cows. Malcolm Beeson, College of Agr., Madison, Wis. Cert. Milk *12*, 137, p. 9, Sept., 1937.

The A. I. V. method of making silage is the preservation of green plant tissue by adjusting the pH to 3 or 4 with mineral acids.

The nitrogen distribution of A. I. V. alfalfa silage showed some proteolysis but only slight ammonia production. The carotene content of this silage was equal to that of the original green tissue.

The feeding of A. I. V. alfalfa silage to dairy cows definitely increased the carotene and vitamin A content of the milk. The blood also showed a much higher carotene content. No unusual changes in milk production resulted from feeding A. I. V. alfalfa silage. Rats fed mineralized A. I. V. silage showed a greater average growth than rats fed mineralized winter milk.

Blood and urine analyses indicated that the higher acid intake of the cows was being neutralized by means which prevented any deleterious effects on the animals. W.S.M.

161. Molasses Silage Produces Unusual Results in Feeding. C. B. Bender, New Jersey Agr. Exp. Station, New Brunswick, N. J. Cert. Milk *12*, 136, p. 9, Aug., 1937.

Feeding trials were conducted to study the color and flavor of milk produced by Guernsey cows when fed on various types of roughage. It was found that the color was maintained more uniformly in the groups receiving grass silage as part or all their roughage. The roughage ration which contained a high proportion of good quality molasses silage was superior to corn silage in its ability to produce milk of a high color and flavor. W.M.S.

162. Need Vitamin A for Normal Reproduction. T. S. Sutton, Ohio Agr. Exp. Sta., Wooster, O. Ohio Agr. Exp. Sta. Weekly Press Bull. *22*, 47, Jan. 27, 1938.

Recent experiments having shown that a ration deficient in vitamin A may produce permanent sterility in both males and females, it is recommended that the practice of feeding bulls low quality hay refused by other classes of livestock be discontinued. W.E.K.

163. Making Silage from Hay Crops. A. E. Perkins, C. C. Hayden, C. F. Monroe, W. E. Krauss, and R. G. Washburn, Ohio Agr. Exp. Sta., Wooster, O. Ohio Agr. Exp. Sta. Bimonthly Bull. *23*, 190, pp. 3–12, Jan.–Feb., 1938.

Four methods of making legume silage (molasses, mineral acid, dry ice, and no treatment) are described briefly. Experiments with a special apparatus for exerting pressure on green material demonstrated that dry-matter content of the material to be ensiled is of prime importance. Appreciable loss of juice was obtained when green material containing less than 25 per cent dry matter was subjected to 8 pounds pressure per square inch. This corresponds to the pressure at the bottom of 26 to 28 feet of legume silage containing 30 per cent dry matter. Samples containing 30 per cent dry

matter lost no juice under 8 pounds pressure and little when under 12 pounds pressure. It is recommended that silo filling operations be so planned that the lighter, dried material go into the silo first and be followed with the heavier, wetter material. W.E.K.

FOOD VALUE OF DAIRY PRODUCTS

164. Vitamin D Milk—Its Rôle in the Prevention and Treatment of Rickets. BENJAMIN KRAMER, Pediatric Research Lab., Jewish Hospital, Brooklyn, N. Y. Cert. Milk *11*, 125, p. 3, Sept., 1936.

The author's conclusions in part are as follows:

The best evidence fails to reveal any difference in potency unit for unit between the different forms of vitamin D milk.

When milks containing a larger number of vitamin D units (400 to 430) are compared with those containing 135 units per quart, the former are found to be more effective as measured by rate of growth, calcium and inorganic phosphorus in the serum, calcium and phosphorus retention in the bones, and complete absence of roentgenological and clinical signs of rickets.

In the prevention of rickets in the prematurely born infant and in the treatment of the disease in the more resistant child, vitamin D milk must be supplemented by other antirachitic agents. W.S.M.

165. Scientific Control of the Nutritional Content of Cow's Milk. W. C. RUSSELL, Rutgers Univ., New Brunswick, N. Y. Cert. Milk *12*, 132, p. 5, April, 1937; *12*, 133, p. 5, May, 1937; *12*, 134, p. 10, June, 1937.

This series of articles deals chiefly with a discussion on attempts which have been made to change the composition of milk by variation of the constituents of the ration. Thirty-four references on the more recent reports on this subject are given. The author concludes that generally speaking, the constituents of milk which give to milk its characteristic properties tend to remain constant in concentration, regardless of the composition of the ration. Thus, the milk proteins, fat, lactose, calcium and phosphorus, tend to be maintained at constant values, whereas vitamins A, C, and D, and iodine can be varied to a considerable extent by the level at which these substances occur in the ration. W.S.M.

HERD MANAGEMENT

166. Calf Raising Problems. H. A. HERMAN, Missouri College of Agric. Jersey Bulletin, *57*, 3, Jan. 19, 1938.

The maintenance of the dairy herd in numbers and established production levels requires the replacement of 20 to 30 per cent of the cattle annually. The necessity for this high replacement rate may be largely attributed to

Bang's disease, mastitis, sterility, and low production. Various reported studies show the average cow in the D.H.I.A. of this country remains in the herd from 3.6 to 4.7 years after freshening. The advantages and disadvantages of raising calves are discussed. It is suggested that in general the most satisfactory method of maintaining and improving the herd is by the raising of carefully selected calves. The calf crop should be culled thoroughly and only those from the best cows and sired by well bred sires kept. Calf mortality is exceedingly high in many herds, studies are reported showing that only about 50 to 75 per cent of all the heifers saved come into production. Scours, pneumonia, and general digestive disturbances account for most of the losses. A plan of careful management and strict sanitary measures is suggested as a means of combatting high calf mortality. The care of the newborn calf, and the raising of the calf to maturity by various feeding programs is discussed. The reader is referred to Mo. Agr. Exp. Sta. Bull. 377 for a description of the various methods successfully employed in calf raising. L.C.

167. What Can be Learned from Production Records. C. Y. CANNON, Iowa State College. Jersey Bulletin *57*, 2, Jan. 12, 1938.

The objectives of cow testing programs are outlined and are stated to be: (1) to obtain a check whereby feeding and management improvements can be made on cows so as to increase financial returns, (2) to discover those superior cows whose blood should be used for the production of the next generation, and (3) to test the breeding powers and merit of bulls in service.

As butterfat production increases from 200 to 500 pounds, the increase in feed costs moves at the rate of about $11 to $12 per cwt. of butterfat. The value of the product increases more rapidly so that there is a greater difference between feed costs and value of product.

Records at the Iowa State College dairy farm shows that of every 100 two-year old heifers entering the herd, 77 per cent become three-year olds; 57 per cent become four-year olds; 41 per cent become five-year olds; 31 per cent become six-year olds; 22 per cent become seven-year olds; 14 per cent become eight-year olds; 11 per cent become nine-year olds. These figures vary but slightly from Iowa Cow Testing Association records. Such losses from the herd are caused by various agents. L.C.

168. Water Your Cows Well. C. C. HAYDEN, Ohio Agr. Exp. Sta., Wooster, O. Ohio Agr. Exp. Sta. Weekly Press Bull. *22*, 44, Jan. 6, 1938.

Cows have a requirement for water from 4 to 5 times greater than the amount provided in their milk. Even dry cows need 10 gallons or more daily. W.E.K.

169. Pasture Improvement Gives Good Returns. D. R. Dodd, Ohio Agr. Exp. Sta., Wooster, O. Ohio Agr. Exp. Sta. Weekly Press Bull. *22*, 47, Jan. 27, 1938.

In a series of tests extending over 7 years, a liberal treatment of 20 per cent superphosphate on limed pasture land produced 244 pounds of beef per acre in comparison with 135 pounds where no phosphate was used. When a high white clover content prevailed the difference was even greater. In terms of milk, the areas without phosphate produced the equivalent of 1078 pounds; all the phosphate areas, an average of 1950 pounds, and the high clover phosphated areas, 2255 pounds. W.E.K.

ICE CREAM

170. Air Conditioning the Retail Ice Cream Outlet. Arthur Behrstock, Walgreen Drug Co., Chicago, Ill. Ice Cream Rev. *21*, 1, p. 22, Sept., 1937.

The experiences of a large drug company who have air conditioned 125 stores is given. The main reasons for air conditioning the stores are: (1) the comfort of the customers, (2) the health and, consequently, the temperament of employees, and (3) the greater cleanliness of merchandise. Best results are obtained when the temperature is not kept too low, and is raised as the outside temperature rises. Relative humidity is kept between 50 and 55 degrees. J.H.E.

171. Automatic Refrigerated Vending Machines. Anonymous. Ice Cream Rev. *21*, 2, p. 31, Sept., 1937.

Refrigerated ice cream vending machines have been developed. The machine described in the article has an ice bunker that holds 18 pounds of dry ice to refrigerate 102 ice cream novelties. The delivery operation is accomplished electrically. J.H.E.

172. Some Attractive Ice Cream Specialties. John Claitor, Birmingham, Ala. Ice Cream Rev. *21*, 2, p. 44, Sept., 1937.

Directions are outlined for making ice cream specialties including fruit pie, baked Alaska, pumpkin pie, fruit cake, ice chocolate cake and nut roll. J.H.E.

173. Some Sanitary Aspects of Ice Cream Making. M. J. Prucha, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. Ice Cream Rev. *21*, 2, p. 74, Sept., 1937.

In the summer of 1936, 480 samples of ice cream collected in different cities in Illinois had an average bacterial count of 1,000,000 per ml. Bacterial analysis of some 600 samples of unpasteurized ingredients added

to ice cream at freezing showed that these ingredients may be a real source of bacteria in ice cream. Other sources of bacteria in ice cream are reviewed. J.H.E.

174. Manufacture of Sherbets and Ices. S. L. TUCKEY, Dept. of Dairy Husb., Univ. of Ill., Urbana, Ill. Ice Cream Rev. *21*, 3, p. 69, Oct., 1937.

The problems involved in the manufacture of ices and sherbets and their corrections are described. J.H.E.

175. Serum Solids—The Rôle They Play in Ice Cream. C. A. IVERSON, Dept. of Dairy Husb., Iowa State College, Ames, Iowa. Ice Cream Rev. *21*, 2, p. 62, Sept., 1937.

Serum solids improve body and texture of ice cream but when used in excessive amounts pronounced defects occur which include, sandiness, sogginess, and poor melting quality. Serum solids may improve the flavor but as the milk solids are increased more flavoring must be added as the additional milk solids serve to subdue the flavoring. Serum solids may be sources of definite "off" flavors. J.H.E.

176. Calculating an Ice Cream Mix Made in a Vacuum Pan. HANS EDEL, Gehl's Guernsey Farms, Milwaukee, Wis. Ice Cream Rev. *21*, 4, p. 34, Nov., 1937.

By use of a chart accompanying the article the author gives instruction for arriving at the quantity of various ingredients to be used in making a mix in the vacuum pan. J.H.E.

177. Ice Cream Floor Plans. Anonymous. Ice Cream Trade J. *33*, 12, p. 8, Dec., 1937.

Floor plans for a 300,000 gallon ice cream plant showing arrangement of rooms and location of the various prices of equipment is presented. A description of the plan of operation is also given. W.H.M.

178. Common Flavor Defects in Ice Cream and How to Control Them. W. C. COLE, Dairy Ind. Div., Univ. of Calif. Ice Cream Trade J. *33*, 12, p. 16, Dec., 1937.

Off flavors in ice cream may be caused by (1) the use of inferior raw materials in the preparation of the mix, (2) the use of inferior flavoring materials in improper combination of flavors, and (3) the development of off flavors during storage. The use of pure vanilla extract rather than imitation vanilla and low storage temperature are recommended. W.H.M.

179. 1936 Ice Cream Production Figures. Anonymous. Ice Cream Trade J. *33*, 12, p. 40, Dec., 1937.

The production of ice cream and sherbet by each state and for all states during 1936 as reported by the U. S. Department of Agriculture is printed in tabular form. The total production of ice cream in 4,441 plants was 248,812,000 gallons representing an increase over 1935. W.H.M.

180. The Association's Year. ROBERT C. HIBBEN, Int. Assoc. Ice Cream Mfgrs., Washington, D. C. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 41, Oct., 1937.

Proposed legislation to be considered by the 1938 legislature and how it would affect the ice cream industry is discussed.

The information service of the association has been increased and numerous requests for publications have come from manufacturers, teachers of home economics and other interested groups.

The Ice Cream Merchandising Institute is disseminating regularly information helpful to distributors of ice cream as well as consumers. M.J.M.

181. Our Industry. MADISON H. LEWIS, Int. Assoc. Ice Cream Mfgrs., The Borden Co., New York, N. Y. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 24, Oct., 1937.

The three major activities of the International Association of Ice Cream Manufacturers at present are the accounting, merchandising, and legislative work.

The accounting bureau completed two general surveys, one an analysis of advertising and the other of sales. The analysis of sales showed a very definite shift in the past years. Bulk sales have decreased, package sales have increased, and there has been a sizable increase in novelty or specialty sales.

The Ice Cream Merchandising Institute has disseminated merchandising hints and materials to association members. Talking slide films have been prepared for retail to ice cream manufacturers for dealer and consumer education. The institute staff also held a series of fourteen merchandising meetings in different parts of the country.

Federal and State legislation which affects the ice cream industry is closely followed with the view of protecting the industry to the greatest extent possible.

Preliminary statistics indicate that the 1937 sales of ice cream will produce the greatest gallonage for any year in the history of the industry. M.J.M.

182. The Dairy Industry's Future. HARRY C. CALVERT, The Pfaudler Co., Elyria, Ohio. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 64, Oct., 1937.

A proposal is made that there be periodic meetings of representatives of the International Association of Ice Cream Manufacturers and Milk

Dealers, together with the Dairy and Ice Cream Machinery and Supplies Association for the discussion of all subjects of a controversial nature. Such discussions should lead to a closer alignment among the three groups in policies and programs affecting the dairy industries' national welfare. M.J.M.

183. Fundamentals of Official Ice Cream Control as We See Them in Birmingham, Ala. L. C. BULMER, Jefferson County Board of Health, Birmingham, Ala. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 66, Oct., 1937.

The fundamentals of ice cream control are: (1) complete pasteurization of all ice cream mix to a temperature of not less than 50° F. for a period of 30 minutes, followed by immediate cooling to the aging temperature; (2) positive control, so far as possible, of ice cream mix after pasteurization so that recontamination does not occur, and (3) improvement on esthetic grounds of the wholesomeness and quality of ingredients entering into ice cream.

The practice of shipping or transporting ice cream mix and freezing it at another place is viewed with considerable alarm by the author. M.J.M.

184. Report of Simplified Practice Committee for 1937. RIDGWAY KENNEDY, JR., Chairman Simplified Practice Committee, Abbott's Dairies, Inc., Philadelphia, Pa. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 88, Oct., 1937.

Sufficient study has been made by the committee to determine that ice cream can be kept in perfect eating condition for 12 to 48 hours in the ice compartment of the household refrigerator. The committee will meet with representatives of the National Electrical Manufacturers Association for the purpose of finding, first, how many types of household refrigerators are so constructed that the ice cube tray shelves are removable, so that ice cream in the present type of bulk and brick containers may be stored in it in quantities from a pint to several quarts. Secondly, the cooperation of manufacturers of household refrigerators will be secured, if possible, in so designing every model that the machines will accommodate the present bulk and factory filled packages.

An increasing number of ice cream manufacturers are adopting the "ice tray type" of package in order to make ice cream available to consumers in a form suitable for storage in household refrigerators of the type having space inadequate for present bulk and brick ice cream packages. M.J.M.

185. Europeans Eat More Ice Cream. SAMUEL H. BAER, Blanke-Baer Extract and Preserving Co., St. Louis. Ice Cream Field *31*, 8, p. 31, Dec., 1937.

Mr. Baer, who recently returned from an European trip, states that in 1923 almost 97 per cent of the ice cream sold in Great Britain was vanilla ice cream. Since that time the volume has increased many fold with an increased percentage of strawberry and ice creams other than vanilla, though vanilla ice cream still comprises over 75 per cent of their volume.

He states that improvement in the quality of ice cream has also taken place in England, especially due to controlled composition of the mixes used, but also because of the use of better flavors as well as improved ice cream machinery from the United States.

In 1930, the author states that there were practically no wholesale ice cream manufacturers in Paris, whereas now there are seven wholesale factories there producing between 50,000 gallons and 500,000 gallons annually. The most modern of these has American equipment. In Switzerland he states that there are now five large ice cream manufacturers. He reports the per capita consumption of ice cream as follows: United States over 3 gallons; Great Britain about one-half gallon; France about 1 pint; and Switzerland about one-half pint. W.C.C.

186. Plea for Uniformity. GLENN M. YOUNG, Missouri State Dept. of Health. Ice Cream Field *32*, 1, p. 9, 1937.

The author states "all health departments, whether state or city, have the same objective—the health of the people. A great deal of confusion, unnecessary duplication and expense could be saved if there were greater uniformity in regulations governing the manufacture and sale of ice cream."

He states that due to the advent of the "counter freezer" ice cream plants present many problems to health officials that are sometimes different from those encountered in milk processing plants. Emphasis is placed upon the necessity of safeguarding the product after pasteurization, since "this is the last sanitary safeguard standing between the final product and the consumer." He continues, "Few public health officials who have the best interest of their people in mind would permit milk to be pasteurized at a plant and then be placed in cans and hauled to drug stores, restaurants, confectionaries or grocery stores for bottling. Exactly this is permitted with ice cream mix. It is even shipped long distances from one state to another. This in itself may not be so objectionable. It is how the mix is handled after reaching these places."

He outlined some of the commonly accepted practices regarding cleaning and care of equipment as a means of safeguarding the product. He points out, however, that, "one of the weakest links in the ice cream chain today is in retailing the product rather than in manufacturing it." W.C.C.

187. Fall Season Suggests New Flavor Recipes. JOHN CLAITOR. Ice Cream Field *31*, 6, p. 47, Oct., 1937.

Suggested recipes for various flavored ice creams suitable for the fall are given. The list includes grape ice, grape meringue and the following ice creams: pumpkin, coconut, persimmon, pear, honeydew, melon, and avocado. W.C.C.

188. Oat Flour as an Antioxidant. SIDNEY MUSHER, Musher Foundation, Inc. Ice Cream Field *31*, 7, p. 31, Nov., 1937.

The author claims that "avenex" (an edible oatflour product) is effective in retarding the development of off flavors in dairy products. To substantiate this claim he refers to previously published results, reproducing some of them in table form. These results also indicate that when 0.5 per cent of "avenex" is used in ice cream that a reduction of at least 25 per cent of the stabilizer content, is desirable in order to avoid overstabilized ice cream. W.C.C.

189. The Matter of Mix. W. H. BROWN, Univ. of Illinois. Ice Cream Field *31*, 3, p. 9, July, 1937; 4, p. 27, Aug., 1937.

The effects of adding various ingredients to ice cream mix after it has been pasteurized was considered in the light of sanitation and the bacterial content of ice cream. Special consideration was given pecan nut meats.

The author reviews some of the work previously published by other investigators to show that although the total number of organisms in the finished ice cream resulting from the addition of nut meats may be small, yet 50 per cent of the samples gave a positive test for *Bacterium coli*.

The author tried 69 different treatments of pecan nut meats but states that only 12 showed possibilities of being successful, and of these only 4 were recommended.

In a comparison of various methods of storing nut meats the author states "The best results from the standpoint of flavor and crispness were secured by storing in an open container at room temperature and the poorest results were secured by storing in an open container in the refrigerator room." These observations were made during the winter months.

Brief mention is also made of the preparation of peaches, bananas, and oranges for use in ice cream.

The following statements are taken from the conclusions drawn by the author:

1. The sanitary quality of pecan nut meats can be greatly improved by dipping in a 50–75 per cent boiling solution of sucrose plus 1 per cent salt followed by drying in a hot air oven. A marked improvement in the flavor of the nut meat usually resulted from such treatment.

2. The treated meats can best be stored in glassine bags at room temperature. The relative humidity should be around 42 to 50.

3. One-tenth of one per cent of sodium benzoate added to coloring materials will not inhibit the growth of bacteria.

4. The addition of 25 per cent alcohol to coloring materials will prevent the growth of bacteria and mold.

5. Coloring materials can be heated to 140°, 160°, or 180° F. for 30 minutes without injuring the quality of the colors. Additional heating, in some cases, slightly reduces the intensity of the color.

6. Strawberries and raspberries can be pasteurized to 145° F. for 30 minutes to improve their sanitary quality when used to flavor ice cream.

7. Peaches mixed with sugar can be boiled for 3 minutes without injuring the flavor of the fruit.

8. The flavor of oranges is not affected when dipped in 75–100 parts per million chlorine water to improve the bacteriological aspects of the fruit for use in ices or sherbets. W.C.C.

190. Scoops as a Source of Contamination of Ice Cream in Retail Stores. ANDREW J. KROG AND DOROTHY S. DOUGHERTY, Health Dept., Plainfield, N. J. Am. J. Pub. Health *27*, 10, p. 1007, 1937.

Bacterial counts of scoop samples were invariably higher than those of samples taken with a sterile spoon indicating surface contamination from scoops. The factors influencing the extent of contamination are discussed. Samples taken with dry scoops were lower than those taken with wet scoops. The authors conclude that if the ice cream scoops and other dispersing utensils are kept on a dry rack protected from flies, dust, and other sources of contamination, instead of in water, and rinsed with either hot or cold tap water after and before each use, the amount of contamination from the dispensing utensils can be greatly reduced. M.W.Y.

191. Does Ice Cream Pay on Retail Dairy Routes? FREDERICK E. JACOB, The Stevens-Davis Co. Milk Dealer *27*, 2, p. 30, Nov., 1937.

The author discusses the pros and cons of ice cream on retail dairy routes. The following conclusions are drawn: If you have a high class product, high class trade with good home refrigeration, reasonably long summer climate, intelligent, aggressive routemen with some bonus incentive to increase their earnings, and not too much cheap package competition, then you should be selling ice cream on your retail routes. But—if you have numerous wholesale routes, less than ten or a dozen routes, too much union domination of routemen, routemen of the old "milk and cream only" school, too short summers, not enough finances to provide the proper refrigeration, a lack of advertising and merchandising facilities, you'd better look twice before you go into ice cream on retail routes. C.J.B.

192. Walgreen's Trains Its Employees. ARTHUR BEHRSTOCK, Ice Cream Trade J. *33*, 8, p. 8, Aug., 1937.

A process, perfected over a period of years, has been developed by the Walgreen Company for training new recruits into a smoothly functioning

army of soda fountain employees. Prospective employees are judged on personal appearance, capacity to learn, and cleanliness. Once an application is approved the prospective employee is required to study a manual designed to help him learn the Walgreen way of doing things. A training course lasting from three days to a week, under the best man in the Walgreen service, is then given, followed by a trip through the commissary where he is impressed with the sanitary methods used in food preparation. An examination ends the employee's formal training. Bulletins telling of new methods are sent out and district meetings are held to discuss common problems. W.H.M.

193. Flavor Drives Build Sales 20 Per Cent. DWIGHT ABBOTT. Ice Cream Trade J. *33*, 8, p. 12, Aug., 1937.

Two weeks' concentration on a single flavor has produced better results than the advertising of many flavors of ice cream. Backbar dominations and colorful window displays have been used successfully by the Pangburn Company of Fort Worth, Texas, to produce increased ice cream sales during the past six months. W.H.M.

194. New Price Policies Pay Big Dividends in Sales. J. EDW. TUFF. Ice Cream Trade J. *33*, 8, p. 16, Aug., 1937.

The Hayden Ice Cream Company, Inglewood, California, has employed a volume dividend price plan to increase sales. Dealers are charged a base price and on the tenth of each month is paid an earned cash dividend based on volume of sales. The company has been able to maintain uniform retail price in its dealers' stores. New dealers are accepted only after a study of the area served by the store, and once the dealer is accepted the company assists him to get started properly by mailing invitations to his customers inviting them to the store for a free pint of ice cream. The dividened scale is based on actual differences in the cost of selling to the different dealers. W.H.M.

195. An Open Letter. MALCOLM PARKS. Ice Cream Trade J. *33*, 8, p. 20, Aug., 1937.

In a letter addressed to the ice cream manufacturer, a dealer calls attention to the many practices originated by the manufacturer which injured the dealer's business. Increased competition for retail stores and other outlets, cheap packages, and other items have only served to make it more difficult for the dealer to make a profit. The dealer states that he has decided to part company with the manufacturer and cooperate with other dealers in promoting their own manufacturing plant, and engage in other activities for the mutual benefit of the group. W.H.M.

196. Refrigeration without Accidents. EDW. R. GRANNIS, Industrial Engineer, Nat. Safety Council. Ice Cream Trade J. *33*, 9, p. 17, Sept., 1937.

Most of the accidents which occur in refrigeration plants are preventable. Proper construction of the building to provide adequate light and ventilation, location of main service switch outside of compressor room, so that electrical current may be cut off in case of emergency, and the isolation of the boiler room from the compressor or generating room by an unpierced fire-resisting wall are recommended. Improper fusion welds on ammonia vessels are sometimes responsible for explosions. A device for the relief of excessively high pressures and the piping of safety valve outlets directly to the outside atmosphere are devised. Some automatic pressure limiting device to stop the action of the compressor at a pressure not higher than 90 per cent of the maximum allowable working pressure is advised. A simple way to detect an ammonia leak is with a stick wrapped in cotton and dipped into a small vial of muriatic acid. The saturated cotton is passed along the suspected pipe lines and fittings, and upon coming in contact with the smallest amount of escaping ammonia, a thick cloud forms above the stick. Gas masks should be available for use and attendants trained in the use of rescue apparatus. Careful operation of all refrigeration equipment by experienced men is of prime importance. W.H.M.

197. Making the Mix in a Vacuum Pan. P. S. LUCAS, Michigan State College, East Lansing, Mich. Ice Cream Trade J. *33*, 9, p. 20, Sept., 1937.

"Advantages claimed for condensing the entire mix are several, such as the use of fresh milk and cream with consequent desirable effects on taste of the mix, not only through the use of fresher products but also by removal of off-flavors through heating in a vacuum." A pan in a combined market milk and ice cream plant offers a means of taking care of surplus milk. The amount of mix made and cost of raw materials are factors which determine whether the purchase of a pan is practicable or not. The cost of condensing the mix varies with capacity, in one plant with 150,000 gallons of mix it amounted to approximately 3 cents per gallon. Directions are given for calculating the amount of ingredients to use when making a mix in the pan. W.H.M.

198. A Guide for Modernization. Anonymous. Ice Cream Trade J. *33*, 10, p. 6, Nov., 1937.

This article is devoted to a discussion of refrigeration equipment. Improvements made in evaporators, compressors, and condensers are described. Improvements in evaporators have been accomplished largely by increasing

the effectiveness of the surface. Modern evaporators are operated "flooded" and air circulation in the hardening room is used to speed up freezing and reduce frost accumulation. Two or more separate compressors provide a very flexible installation, and a booster compressor is a very effective method of handling low pressure vapor. Floor space can be conserved through the use of V-belt drives. Emphasis is placed on the desirability of designing a plant so that the highest possible suction pressure can be used, consistent with temperatures required.

A good condenser should have effective heat transfer surface and facilities for easy cleaning. Attention should be given the condensing water supply. The coldest water available consistent with cost should be used. Provision for removing non-condensible gas is necessary. An automatic purger connected to the top of the receiver is superior to hand purging and will usually pay in a short time by reducing power consumption. W.H.M.

199. Oat Flour as an Antioxidant in Ice Cream. W. S. Mueller and M. J. Mack, Mass. State College, Amherst. Ice cream Trade J. *33*, 10, p. 24, Oct., 1937.

"The results secured in this experiment confirm previously published work which showed oat flour to have antioxidative properties when used in ice cream. The use of only 0.25 per cent of oat flour in the mix delayed the development of off-flavors during storage of the resultant ice cream, although 0.5 per cent proved more effective.

"Oat flour also has the properties of a stabilizer. The stabilizing action of the oat flour increased mix viscosity, improve the body and texture, and increased the melting resistance of ice cream. When oat flour is added to the mix, a reduction should be made in the amount of gelatin or other stabilizer used if an overstabilized condition is to be avoided. The results indicate that a reduction of at least 25 per cent of the gelatin content is desirable when 0.5 per cent oat flour is incorporated in the mix." W.H.M.

200. The Rôle of Eggs in Ice Cream. W. H. Martin, Kansas State College, Manhattan. Ice Cream Trade J. *33*, 10, p. 29, Nov., 1937.

A review of the literature dealing with the use of eggs in ice cream is presented. Advantage gained from the use of eggs, kinds of eggs available, amounts and method of using eggs are some of the topics discussed. W.H.M.

Other abstracts of interest are numbers 125, 127, 132, 133, 134, 135, 136, 145, 148, 151, 157, 160, 164, 201, 202, 207, 208, 209, 212, 215, 216, 217, 218, and 219.

MILK

201. The Lesser Known Constituents in Milk. G. Genin, Paris, France. Le Lait *17*, 168, p. 820, Sept.–Oct., 1937.

For 100 molecules of fatty acid combined in the form of triglycerides in the fatty matter of milk, there are 25 to 37 molecules of oleic acid, 24 to 27 molecules of palmitic acid, while butyric, myristic and stearic represent 7 to 10 molecules each. The other acids which are not very clearly defined consist of lauric, caproic, caprylic and capric. Milk fat also contains a small proportion, generally less than 1 per cent, of polyethenoidic acids of 20 or 22 carbon atoms in their molecule. The fact that the completely saturated acids are high in comparison with the contents of these acids in other natural fats has led the author to think that in milk fat the glycerides of the lower fatty acids are formed from oleoglycerides reformed by a combined phenomenon of oxidation and reduction. When cod liver oil is added to the feed of cows, certain of the polyrthnoidic acids characteristic of that oil, pass into the milk fat which then contains 5 to 7 per cent of these acids. At the same time the yield of fat in the milk is reduced and its composition is profoundly affected. The proportion of lower fatty acids is reduced to about one-fourth their normal value, the content of palymitic, stearic, and myristic acids is likewise reduced but the content of oleoglycerides is only slightly modified. These changes in the composition of the fat are only temporary and when cod liver oil is removed from the feed the fat composition becomes normal again.

Milk possesses the power to reduce methylene blue even if it contains none or few bacteria. Skimmilk does not reduce methylene blue as the reducing substance appears to have been removed by entrainment in the cream during separation. Holding the milk at 63° C. (145.4° F.) for 30 minutes destroys the enzymes or other substances responsible for the reduction of methylene blue. Raw cream diluted with water, reduced methylene blue. A number of substances were studied for the power to reduce methylene blue. Of the substances studied only aldehydes and hypoxanthine showed definite reducing activity to methylene blue. Attempts were made to separate the reducing substances from raw milk or cream by dialysis. Negative results were obtained but after dialysis certain of the enzymes in milk became inactive. Ascorbic acid was shown to play but an insignificant rôle in the anaerobic reduction of methylene blue.

The lipase activity in milk was determined in a buffer mixture at pH 8.5 using tributyrin as the substrate. The liberated butyric acid was removed by steam distillation and titrated. The lipase activity of milk showed considerable variation from one animal to another and from one stage of lactation to another. Lipase in milk is more readily destroyed than phosphatase. There is more lipase matter in the milk serum than in the fatty matter while the opposite is true of phosphatase. It is probable that there is no relation between the lipase and phosphatase activity of milk, the latter probably being associated with the efficiency of the mammary gland. Difficulties encountered in determining the catalase activity of milk are discussed and a review of applicable methods is given. A.H.J.

202. The Effect of Light on the Vitamin C Content of Milk. S. K. Kon and M. B. Watson, Nat. Inst. for Res. in Dairying, Univ. of Reading, England. Biochem. J. *30*, 2273, 1936.

The vitamin C content of milk was determined by the titration procedure using 2–6 dichlorophenolindo-phenol reagent. Evaluations were made before and after treatment with H_2S, permitting measurement of vitamin C as reduced ascorbic acid, and, reduced and reversibly oxidized forms (total ascorbic acid) respectively.

Milk giving a positive chemical test for vitamin C fails to reduce the indophenol reagent (reduced form of ascorbic acid absent) after exposure to 1 hour February sunshine while contained in glass. The reducing power can be partially restored by use of H_2S (oxidized form produced) but losses always take place.

The reaction shown above is due mainly to visible radiation of short wave length (blue and violet) although ultra violet radiation is also probably active. Visible radiation of longer wave lengths (yellow and red) are without effect.

When the dissolved oxygen of milk (in glass) is completely replaced by an inert gas, exposure to sunlight for 2 hours is without effect upon the reaction. Pasteurization had little effect on the influence of light on the ascorbic acid values of milk as determined by the titration procedure.

When milk is exposed to sunshine of sufficient intensity, the effect of light on vitamin C may be observed in two separate reactions: (1) a reversible change of the ascorbic acid to a substance (reduced into reversibly oxidized form) no longer able to bleach the indophenol reagent unless treated with hydrogen sulphide; to effect this change both light and oxygen are essential and this reaction does not proceed in the dark, and (2) a more gradual change of the product of the first reaction to a substance which cannot be caused by H_2S treatment to give a positive test with indophenol and which is biologically inactive. The latter product is suggested to be 2 : 3 diketo-1-gulonic acid.

A pint bottle of milk exposed to sunlight on the doorstep for half an hour and then kept for 1 hour in the dark loses fully half its original antiscorbutic properties. Pasteurization by the holder method does not affect the reduced form, but destroys the reversibly oxidized form, of ascorbic acid in milk.

K.G.W.

203. Cream Plug—What Can be Done to Avoid It. Joseph Burns, Capitol Dairy, Madison, Wisconsin. Milk Dealer *27*, 3, p. 92, Dec., 1937.

The author discusses his experience with cream plug and draws the following conclusions:

To eliminate cream plug: Avoid excessive agitation. Heat and cool quickly to have short agitation time. Agitator should not go more than 70

r.p.m. Avoid excessive heating. Temperature in pasteurization of cream should not go above 150°. Avoid incubation temperatures. Cool cream immediately after separating if cream is to be held for any length of time prior to pasteurization. C.J.B.

204. The Use of Vending Machines in the Market Milk Industry. JAMES R. HUDSON, Baker-Hubbel Dairy, Peoria, Ill. Milk Dealer *27*, 3, p. 32, 48, Dec., 1937.

The author discusses the advantages and disadvantages of dispensing milk through vending machines in factories, schools, and office buildings. C.J.B.

205. New Type Conveyor Switches Effect Substantial Savings in Modernization Program. Milk Dealer *27*, 3, p. 30, Dec., 1937.

A description of how the installation of combiner and selector type divider switches in connection with bottle conveying installations effected a substantial saving in equipment, space requirement, and production costs at the Jansen Dairy, Hoboken, New Jersey. C.J.B.

206. Sale of "By Products" Growing Factor in Milk Industry. Milk Dealer *27*, 3, p. 29, Dec., 1937.

A survey by the Milk Dealer showed that almost 87 per cent of the dealers replying to a general questionnaire reported that they sold chocolate milk, 93 per cent advised that they handled buttermilk, 67 per cent sold orange juice, 80 per cent sold cottage cheese, 67 per cent sold butter, and 45 per cent handled eggs.

Chocolate milk accounted for 2½ per cent, buttermilk for 5 per cent, orange drinks for 3.8 per cent, and tomato juice for 0.7 per cent of the total volume of sales of those dealers reached by the questionnaire. The sale of tomato juice decreased while buttermilk and cottage cheese sales increased during the past two years. C.J.B.

207. The Structure and Composition of Foods, Vol. 3, Milk, Butter, Cheese, Ice Cream, Eggs, Meat, Meat Exracts, Gelatin, Animal Fats, Poultry, Fish, Shellfish. ANDREW L. WINTON AND KATE BARBER WINTON. Published by John Wiley and Sons, 1937, pp. 524, Price $8.00.

This book is the third of a series of four books dealing with the structure and composition of foods. The first 209 pages are devoted to the structure and composition of milk and milk products, the subjects covered in this review.

Apparently the authors have endeavored to compile the important contributions in the literature showing the composition of milk and milk prod-

ucts from the viewpoint of the analytical chemist, and to present them in an organized manner to give a systematic treatise of the subject. The many references cited are given as footnotes on each page. There has been little effort made to give a complete statement regarding each phase of the chemistry of milk and milk products but rather to quote the literature. The book has its chief value as a literature review and the reader should be able to interpret certain statements and expand some of the text to secure a true impression of the facts. For example, the paragraph on "microscopic characters" of human milk states that the fat globules in human milk are smaller than those in cow's milk but fails to specify their size except that they are less than 10 μ. In the section on the fat globules in cow's milk their size is not definitely stated but data presented show them to vary "from less than 1 to over 20 μ, the average, according to L. L. Van Slyke, being over 2.5 μ."

This book will be of value primarily as a handy reference to secure some of the important publications pertaining to a special aspect of the composition of milk and milk products. A.C.D.

208. The Reliability of Flavor Judgments, with Special Reference to the Oxidized Flavor of Milk. G. MALCOLM TROUT AND PAUL F. SHARP, Cornell Univ. Agr. Exp. Sta., Ithaca, N. Y., Memoir 24, June, 1937.

A total of 1207 ten-sample series, representing 12,070 taste judgments, of sodium-chloride, sucrose, lactose, lactic-acid, and quinine-sulfate solutions were judged in these studies. Judgment was made also on 3687 additional samples of sodium-chloride and sucrose solutions.

The temperatures at which the maximum discriminatory ability for the respective solutions was found, were as follows: sodium-chloride, 21° C.; sucrose, 35°; lactose, 35°; lactic-acid, 21°; quinine-sulfate, 21°.

The sense of taste was found to be capable of discriminating as low as 1 per cent changes in concentration of the sodium-chloride solutions ranging in concentration from 0.13 to 0.20 per cent. With the sodium-chloride, sucrose, lactose, lactic-acid, and quinine-sulfate solutions, 10-per cent changes in concentration were readily detected.

The amount of substance required to produce a noticeable change in sensation was found to be but a very small percentage of the usually stated value.

The amount of retasting necessary before arriving at final judgment depended upon the concentration, its range, and the number of samples within the series. In ten-sample series of weak solutions, tasting some samples as many as ten times was found necessary.

The mean time, based upon approximately 250 trials each, required for four judges to place a ten-sample series of various solutions, was 4.4, 4.4, 6.1,

and 5.3 minutes, respectively. The mean time in placing the 1057 series was 5.04 minutes. No correlation was found between the time naturally required to place the series, and the accuracy of the judges.

After having placed a ten-sample series in order of concentration ten times, the judges had the samples sufficiently in mind to enable them to select any sample from the shuffled series and by taste alone name its concentration with remarkable accuracy. Graphs are presented showing the percentage distribution of these single-sample judgments of individual samples of several series by four judges. The mean differences between the true concentration and the estimated concentration, for solutions of sodium chloride selected at random from ten-sample series, were 0.029, 0.028, 0.019, and 0.042 per cent, respectively, for the four judges. The mean difference for 1987 individual samples tested was 0.029 per cent.

Retasting forty-sample series of sodium-chloride solutions five times improved the correlation from approximately 0.84 to 0.99. The final arrangement of a series was accomplished through end selection.

Samples of milk totaling 2152, involving 8608 taste judgments, were studied for the oxidized flavor.

The consistency of six commercial-milk judges in re-scoring milk was determined. The comparison between the percentage of all samples scored within a narrow range of scores, and the percentage of all samples re-scored with no deviation, seemed to give a more accurate indication of the consistency of scoring than did the percentage of identically re-scored samples alone. E.S.G.

209. The Handling of Milk and Milk Products. A. T. R. MATTICK, Nat. Inst. for Res. in Dairying, Univ. of Reading, Ministry of Agriculture and Fisheries. Bul. 31, pp. 101, London, England. Obtainable from British Library of Information, 270 Madison Avenue, New York, Price 65 cents.

The fifth edition of this bulletin devotes considerable space to clean milk production and in addition gives an account of certain hitherto unpublished work carried out at the National Institute of Research in Dairying.

The essentials of clean milk production are presented in a clear and concise manner. Consideration is given to the cleanliness of farm workers, care of udder, method of milking and washing and sterilization of utensils and apparatus. The experimental data given relative to sterilization of equipment show the general superiority of proper steam sterilization over the use of disinfectants.

The hitherto unpublished research carried out at the Institute and presented in this bulletin includes sections on pasteurization, new media for the examination of milk, defects and off flavors in dairy products and the nutritive value of milk. Consideration is given to a comparison of the

nutritive value of raw, pasteurized and sterilized milk, the effect of pasteurization on vitamins and the effect of pasture and winter feeding on vitamins. J.C.H.

210. Observe Changes in Ohio Milk Markets. R. W. SHERMAN, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. *22*, 50, Feb. 17, 1938.

During the past 10 years there has been a definite upward trend in butterfat content of milk delivered by 2500 shippers in four Ohio fluid milk markets—Canton, Cincinnati, Columbus, and Dayton. In Columbus and Dayton the average sales per day per dairy has increased. This is thought to indicate a reduction in the number of small producers. W.E.K.

211. Production of Vitamin D Milk Increasing. W. E. KRAUSS, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. *22*, 9, Feb. 10, 1938.

It is estimated that about 3 per cent (400,000,000 quarts) of the fluid milk sold in the United States during 1937 was vitamin D milk. In 5 years the production of vitamin D milk has been comparable to that of pasteurized milk production during the first 5 years following its inception. W.E.K.

212. Pasteurization in England and America. Editorial. Am. J. Pub. Health *27*, 9, p. 920, 1937.

In England, pasteurization has not received as much recognition as in this country although strongly advocated by leaders in the British medical and public health professions. Figures are given which indicate that market milk in large cities is much safer than in rural sections. Recent investigations at the National Institute for Research in Dairying at Reading, in Great Britain, have confirmed the fact that pasteurization has no appreciable effect upon the excellent nutritive qualities of milk. M.W.Y.

213. Fermented Beverages from Milk. ANTONIN MOULIN. Le Lait *17*, 169, p. 946, Nov., 1937.

Details are given for the making of Kefir, Yoghourt, and Koumiss. A.H.J.

214. More Ammonia in Sour than in Fresh Milk. A. E. PERKINS, Ohio Agr. Exp. Sta., Wooster, Ohio. Ohio Agr. Exp. Sta. Weekly Press Bull. *22*, 45, Jan. 13, 1938.

Using an improved method developed by the author, it was found that whereas fresh milk contains only 3 to 5 parts of ammonia per million, sour milk may contain 20 or more times this quantity. This is probably due to the action of bacteria on the proteins of the milk. The amount of ammonia

in milk was not greatly changed by pasteurization or by storing and handling under proper conditions. W.E.K.

Other abstracts of interest are numbers 123, 124, 125, 126, 127, 128, 132, 134, 135, 144, 145, 148, 149, 151, 153, 156, 157, 158, 159, 160, 164, 165, 182, 183, 188, 196, 199, 215, 216, 217, 218, and 219.

MISCELLANEOUS

215. Minimum Wage Legislation. BERNARD SUMMER, Member of the New York Bar. Ice Cream Trade J. *33*, 12, p. 36, Dec., 1937.

A table is printed to show the powers of the Wage and Hour Commissions which have been established in various states adopting such legislation. In future articles, employee information, powers of the wage board, and violation and penalties will be tabulated. W.H.M.

216. The Employer's Job. WHITING WILLIAMS, Cleveland, Ohio. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 29, Oct., 1937.

This is a discussion of employer-employee relationships. Some problems of the employee are set forth, an understanding of which is desirable for co-operative relationships between employer and employee. M.J.M.

217. What's Around the Corner. GUSTAVUS W. DYER, Vanderbilt Univ., Nashville, Tenn. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 51, Oct., 1937.

The speaker presented his views of the functions of business and government. M.J.M.

218. Our Economic Interdependence. H. W. SUMNERS, Member of Congress, Dallas, Texas. Proc. 37th Ann. Conv. Int. Assoc. Ice Cream Mfgrs. Vol. 1, p. 80, Oct., 1937.

The principal ideas expressed in this address are that the success of our government is based on the help and cooperation of the citizens of the country and likewise, success of any specific industry is largely dependent on the successful operation of industry in general. M.J.M.

219. Present-Day Tax Problems. C. A. JAY, Vice-Pres., Industrial, Commercial and Agricultural Conference, Dallas, Texas. Proc. 37th Ann. Conv. Int, Assoc. Ice Cream Mfgrs., Vol. 1, p. 72, Oct., 1937.

The present tax situation is set forth and it is urged that tax payers give more attention to the broad problem of taxation and public expenditures. M.J.M.

PHYSIOLOGY

220. Increased Sodium Chloride Appetite in Pregnant Rats. BRUNO BARELARE, JR., AND CURT P. RICHTER, Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore. Am. J. Physiol. *121*, 1, p. 185, Jan., 1938.

The sodium chloride appetite of twelve female rats during pregnancy was studied by the method of self-selection. On the average, animals ingested over twice as much sodium chloride solution (3 per cent) in the ten-day period after conception as in the ten-day period before conception, and ingested over three times as much in the second half of the period of gestation. The sodium chloride intake in the first ten days postpartum fell back to a level only slightly above the pregestational level. It has been reported that during pregnancy there is a diminution in both anion and cation content of the blood. Among the cations, the sodium content suffers the greatest reduction, whereas among the anions the serum bicarbonate, serum protein, and organic acid are reduced. It appears hightly probable that the animal in some way reacts to this reduction in electrolytes by voluntarily ingesting more sodium chloride.

Other changes in appetite probably occur during pregnancy. Further experiments are now being conducted with regard to this point. D.L.E.

221. The Experimental Production of Severe Homogeneous Osteoporosis by Gastrectomy in Puppies. R. A. BUSSABARGER, SMITH FREEMAN AND A. C. IVY, Dept. of Physiology and Pharmacology, Northwestern University Medical School. Am. J. Physiol. *121*, 1, p. 137, Jan., 1938.

The stomach is essential for the normal growth and development of the bony skeleton of puppies. No evidence of rickets was observed in any of the roentgenograms made of these animals. The serum calcium, phosphorus, and phosphatase of the gastrectomized puppies were usually within the normal range. The deficient ossification of the bones is apparently due to a combination of three factors, namely, (a) the absence of hydrochloric acid which normally renders the less soluble calcium salts more soluble and assists in the maintenance of an acid reaction in the intestine; (b) the absence of the reservoir function of the stomach which increases the speed of intestinal transport of food substances; and (c) the presence of an "acid tide" after eating which tends to decrease calcium retention.

Although soluble calcium salts can be absorbed, two possible etiological factors remain. One is that food calcium is inadequately absorbed. The other is that after the calcium is absorbed, it is inadequately retained. In the absence of hydrochloric acid, organic acids such as carbonic and lactic acid and possibly the bile acids, assist in the absorption of calcium. Gastrec-

tomized dogs also manifest a hyperplasia of the bone marrow, which may contribute to the production of osteoporosis. D.L.E.

222. A Study of Protein Anabolism and Catabolism on a Nitrogen-Free Diet. WALTER H. SEEGERS, Samuel S. Fels Research Institute, Antioch College, Yellow Spring, Ohio. Am. J. Physiol. *121*, 1, p. 231, Jan., 1938.

When rats are given a nitrogen-free diet during pregnancy the N for the young is transferred to them from the maternal reserves. If there is any wastage of N in this transfer, as a result of the young selecting a different assortment of amino acids than is liberated from the maternal reserves, it should appear in the urine. The newly formed tissue of the young will also have a catabolic phase of metabolism yielding N. The combined effect of wastage due to transfer of amino acids and that due to the catabolism of the young was measured and found to be small.

Pregnant animals have a much higher urine N coefficient than non-pregnant control animals, which suggests a higher metabolic rate. After the animals had given birth to young and had used a large quantity of their reserve protein they were continued on the diet until they died. At death the animals had lost 59.08 per cent of their original body weight and 58.42 per cent of their original N supply. As much as 64.4 per cent of the normal protein supply of the animal can be used before it succumbs, and the loss in body weight is of the same order of magnitude. D.L.E.

223. Studies on the Pancreas and Liver of Normal and of Zinc-Fed Cats. D. A. SCOTT AND A. M. FISHER, Connaught Laboratories, University of Toronto, Toronto, Canada. Amer. J. Physiol. *121*, 1, p. 253, Jan., 1938.

The authors mention that previous work has shown the presence of zinc in commercial preparations of insulin. More recently it has been demonstrated that the presence of minute quantities of zinc in suspensions of protamine and insulin not only increases the stability of these preparations but also prolongs their blood-sugar lowering effect. Although zinc is a necessary constituent of the diet for the normal growth of mice and rats and is present in all tissues and organs of the body in amounts almost as great as that of iron, its physiological function has not as yet been clearly demonstrated.

In trials conducted, cats fed on a high zinc diet (.25–.30 gram zinc oxide per day) for from 12 to 16 weeks showed a loss in weight averaging about 900 grams per cat. The amount of zinc per gram of pancreas and of liver was about 7 and 15 times, respectively, as great as that found in similar organs in the control group of cats. The total insulin content of the pancreas was not disturbed. Marked fibrotic changes in the pancreas of all the cats on a high zinc diet were observed. D.L.E.

224. The Adrenals and Gonads of Rats Following Thyroidectomy Considered in Relation to Pituitary Histology. ISOLDE T. ZECKWER, Dept. of Pathology, School of Medicine, University of Pennsylvania, Philadelphia. Am. J. Physiol. *121*, 1, p. 224, Jan., 1938.

As a result of thyroidectomy in young rats, there is retardation in kidney growth and increased weight of the pituitary in both sexes. In males there is no significant change in absolute weights of adrenals and testes, but the ratios of adrenals to kidneys, and the ratios of testes to kidneys are significantly increased. In females there is a decrease in absolute weights of the adrenals and gonads but this is proportional to retardation in kidney growth, so that the ratio of adrenals to kidneys and ovaries to kidneys are not significantly altered. Acidophiles and thyroidectomy cells can possibly be excluded as elaborators of adrenotropic hormone. By a process of exclusion, it is concluded that adrenal growth may depend upon a basophile cell.

D.L.E.

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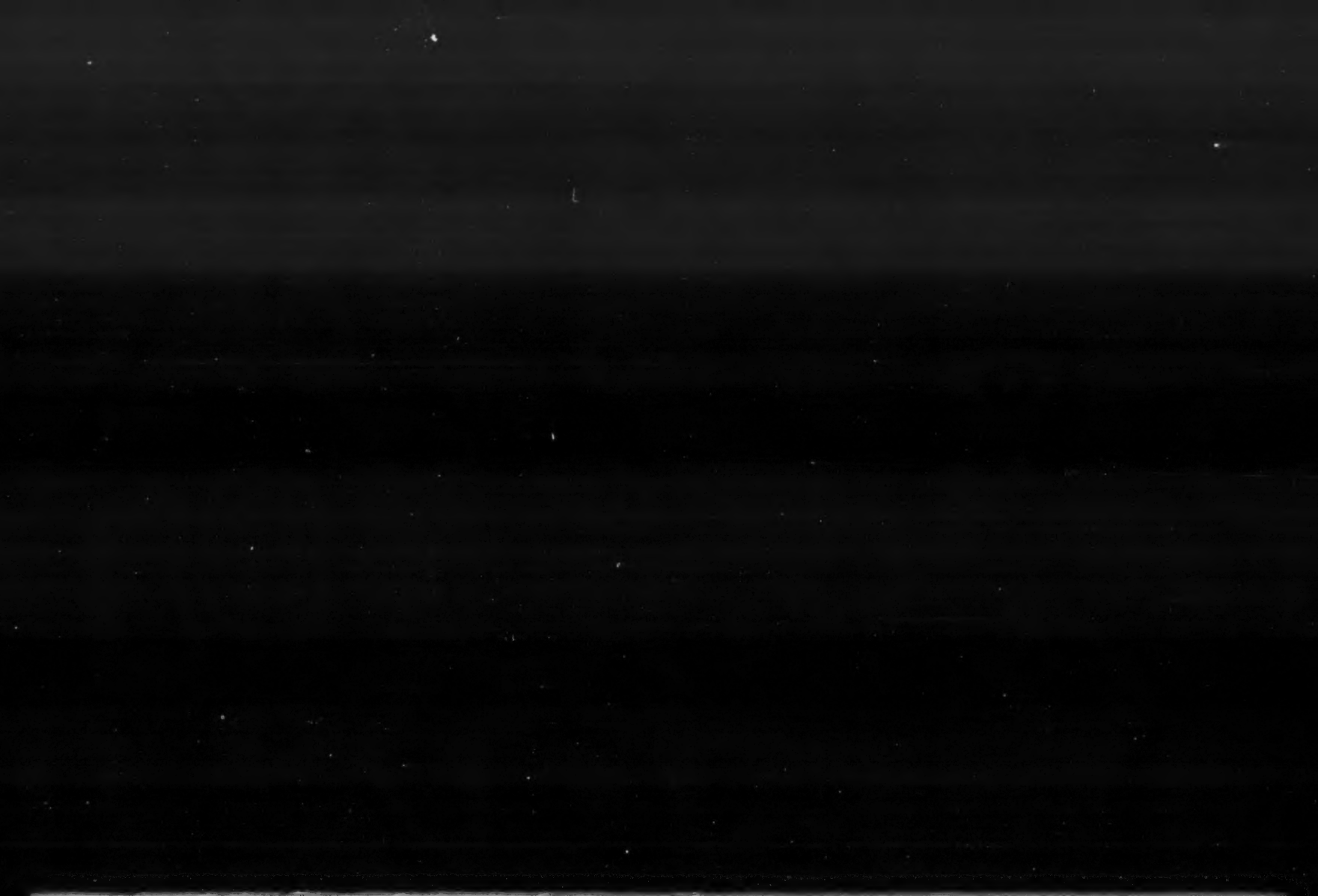

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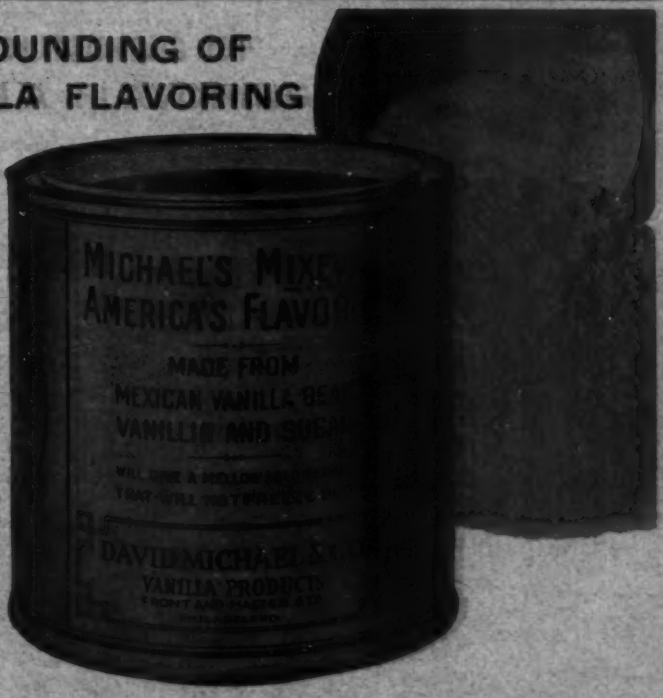

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